Integrative Approaches for Conservation Management of Critically Endangered Nassau Grouper (*Epinephelus striatus*) in The Bahamas

Submitted by
Krista Danielle Sherman
to the University of Exeter as a thesis for the degree of
Doctor of Philosophy in Biological Sciences
February 2018

I certify that all material in this thesis which is not my own work has been identified and that no material has previously been submitted and approved for the award of a degree by this or any other University.

(Signature) ……

Abstract

Species conservation is typically founded upon a range of management strategies, which integrate both biological and socioeconomic data. In this thesis, population genetics, acoustic telemetry, spawning aggregation surveys and stakeholder assessments were used to address key knowledge gaps limiting effective conservation management for critically endangered Nassau grouper (*Epinephelus striatus*) stocks in The Bahamas. A panel of polymorphic microsatellite markers was optimised to assess the genetic population dynamics of more than 400 Nassau grouper sampled throughout the country. Microsatellite data indicate that contemporary Nassau grouper populations in The Bahamas are predominantly genetically diverse and weakly differentiated, but lack geographic population structure. Assessments of changes in effective population size (N_e) show substantive reductions in N_e within The Bahamas compared to historic values that are likely due to natural disturbances. Evidence for recent bottlenecks occurring in three islands as well as an active spawning site, along with higher inbreeding coefficients in two islands were also found, and can be attributed to more recent anthropogenic activities. Collapse of a historically important Nassau grouper fish spawning aggregation (FSA) was supported by both acoustic telemetry and spawning aggregation survey dives. Restriction-site-associated DNA sequencing (RAD-seq) of 94 Nassau grouper was used to explore intraspecific population dynamics, loci under selection and patterns of gene flow in The Bahamas. Genomic assessments of diversity were in accord with microsatellite data and examinations of gene flow support higher levels of connectivity in The Bahamas than was previously suggested. The increased resolution gained from assessments of genomic data support intraspecific population structuring that may be driven by differences in gene flow and putative loci under divergent selection. Telemetry data were successfully used to identify the origins of spawning adults, and support demographic connectivity through migrations between an active FSA in the central Bahamas and home reef habitats within the Exumas and a no-take marine protected area. Stakeholder assessments highlight the complexities of fisheries management within The Bahamas, with key stakeholders often exhibiting conflicting opinions regarding the status of Nassau grouper and the efficacy of

management options. However, these groups mutually agree upon the need to better manage remaining Nassau grouper stocks within The Bahamas through science-grounded policies. Synthesis of these studies along with a review of fisheries governance in The Bahamas was used to develop a comprehensive national management plan for Nassau grouper to facilitate better conservation for remaining populations of this ecologically important marine species.

Table of Contents

Appendices

List of Tables

Chapter I

Chapter II

List of Figures

Chapter I

Chapter II

Chapter III

Chapter IV

Chapter V

Chapter VI

List of Accompanying Material

Acknowledgements

The last three years have been exceptionally challenging, but rewarding. I am extremely grateful to my family (especially my mom, Veronica and sister, Keri) for your unwavering love, emotional and financial support. I would not have made it through the highs and lows of this experience without you.

To my main supervisor, Professor Charles Tyler, thanks for answering my email five years ago and agreeing to take me on as a PhD student. I am immensely appreciative of this opportunity and for the suite of new skills acquired during the course of my PhD. Your extensive knowledge and insight has helped to improve my work and I admire your dedication to research and passion for the environment. Dr. Jamie Stevens, thank you for adopting me into the Molecular Ecology and Evolution lab group (MEEG), as I truly benefited from the experience. I will miss our planned and impromptu discussions and your mischievous sense of humour. Dr. Steve Simpson thanks for showing me around Geoffrey Pope during my first week and for your positive enthusiasm over the years.

Thank you to my friends at home in The Bahamas and abroad for your patience, understanding and encouragement – particularly, Lindy, Karen, Cristina and Craig. I enjoyed my time in Exeter, writing in Lab 201 and completing molecular work with colleagues in the MEEG. Andy, thanks for your patient instruction in the lab and guidance with data analysis. Who knew an Evanno plot could create such a strong reaction? Massive thanks to my sister from another mister and STACKS wiz, Josie, and R guru, Tom for your help during the last few months while I was struggling through various RAD-seq analyses. To John and Anke, thank you for keeping lab 201 going. Josie, Amy, Shelly, Jane, and Sara I am grateful for your friendship and will miss our discussions, coffee/tea breaks, late night work sessions, random kitchen dances, and karaoke evenings.

Special thanks to my talented cousin, Jonathan Cartwright for creating the wonderful Nassau grouper illustrations utilised in the management plan and in this thesis. I would also like to extend my heartfelt gratitude to the following people whose financial contributions enabled me to complete my PhD: Veronica Moncur-Sherman, Keri Sherman, Ignacio de La Rocha, Stacey Moultrie, Royston Jones, Aaron Shultz, Edward Moncur, Carmine and Melonie Ciccollela, Mary Grey, Alexis

Grey-Munnings, Lindy Knowles, Megan Kelly, Regina Smith, Neal Watson and Oihane Diaz de Cerio. Research funding and in-kind contributions have been acknowledged at the end of each chapter along with names of the numerous volunteers that assisted with fieldwork and sampling.

Finally, I wish to dedicate this research in loving memory of two amazing people: my amazing cousin, Lexi and to Keith, a wonderful colleague and friend.

Author's Declaration

I, Krista Danielle Sherman completed the following work presented in this thesis:

Chapter: I wrote and published this review paper with constructive feedback from co-authors.

Chapter II: I designed and conducted fieldwork, coordinated sample collection, performed genomic DNA extractions and quality testing, primer optimisation, genotyping, microsatellite data analysis and wrote the manuscript. Input from co-authors and reviewers helped to improve the paper.

Chapter III: I conducted fieldwork and wrote the paper along with co-authors. Data was analysed by K. Stump.

Chapter IV: I designed and executed fieldwork, performed genomic DNA extractions and quality testing, prepared samples for RAD-tag library development, analysed the data and wrote the manuscript along with co-author contributions.

Chapter V: I designed stakeholder questionnaires, analysed the data and wrote the paper. Input from Professor Charles Tyler and two reviewers helped to improve the paper.

Chapter VI: I conceived of this review paper along with K. Murchie and A. Shultz. The introduction, Nassau grouper, part of the MPA section and the conclusion was written by me and include co-author edits. I also analysed the data for this manuscript.

Chapter VII: This chapter represents a summation of the research and was written by me with feedback from Dr. Jamie Stevens and Professor Charles Tyler.

Abbreviations

AD	Anno domini
BLAST	Basic aligner search tool
BNPAS	Bahamas National Protected Area System
BNT	Bahamas National Trust
bp	Base pair
CEI	Cape Eleuthera Institute
CI	Confidence interval
cm	Centimetre
CUs	Conservation units
DAPC	Discriminate analysis of principal components
ddRAD-seq	Double digest restriction-site-associated sequencing
DMR	Department of Marine Resources
DNA	Deoxyribonucleic acid
ECLSP	Exuma Cays Land and Sea Park
eDNA	Environmental DNA
ELISA	Enzyme-linked immunosorbent assay
ESA	Endangered Species Act
ESU	Evolutionary significant unit
FSA	Fish spawning aggregation
F_{IS}	Coefficient of inbreeding
F_{ST}	Fixation index
ha	hectare
H_E	Expected heterozygosity
H_O	Observed heterozygosity
HSPs	Heat shock proteins

HWE	Hardy-Weinberg equilibrium
IAM	Infinite Alleles Model
IAS	Invasive alien species
IBD	Isolation-by-distance
IUCN	World Conservation Union
km	Kilometre
MAF	Minor allele frequency
MCMC	Markov Chain Monte Carlo algorithms
mi	Mile
ML	Machine learning
MPA	Marine protected area
mtDNA	Mitochondrial DNA
MU	Management unit
N_e	Effective population size
NGO	Non-governmental organisation
PCoA	Principal coordinate analysis
PCR	Polymerase chain reaction
QTL	Quantitative trait loci
RAD-seq	Restriction site Associated DNA sequencing
SD	Standard deviation
SL	Standard length
SNP	Single nucleotide polymorphism
TL	Total length
TURFs	Territorial user right fisheries
µl	Microliter
wk	Week

Research Aims & Objectives

The overarching aim of this research was to apply complementary approaches to evaluate the population health of Nassau grouper (*Epinephelus striatus*) within the Bahamian archipelago and to use the outputs to develop robust scientific recommendations to facilitate better conservation management. The research presented in this thesis has been comprised of seven chapters, including two review papers (Chapters I and VI) and four data chapters (Chapters II, III, IV and V). Chapter I provides a synopsis of Nassau grouper including key knowledge gaps and offers a suggested framework for directing research that could be applied to better conserve the species. The subsequent data chapters utilise molecular (i.e. microsatellite and restriction-site-associated DNA sequencing (RAD-seq)) analyses, *in situ* monitoring techniques (e.g. diver surveys and acoustic telemetry), as well as assessments of commercial fishery landings data and stakeholder perspectives to address questions outlined in Chapter I. Specifically, the following objectives and hypotheses have been addressed:

1. Assess genetic diversity, population structure and effective population size (Chapters II, IV)

H_1: Subpopulations of Nassau grouper do not exist in The Bahamas.

H_2: Estimates of N_e do not give cause for concern.

H_3: Population bottlenecks have not occurred within The Bahamas.

H_4: The genetic composition of Nassau grouper has not been compromised.

2. Describe migratory patterns and assess the state of Nassau grouper FSAs (Chapters III, IV)

H_1: Migration patterns of Nassau grouper follow the shelf edge.

H_2: Migration timing and distances travelled by Nassau grouper are consistent with published studies.

3. Investigate stakeholder perspectives on the status of the Bahamian Nassau grouper fishery (Chapter V)

The research presented in this chapter, was not hypothesis driven, but based on a need to quickly evaluate key stakeholder views on the status of the species and to incorporate their input into the management plan. We anticipated that there might be differences between groups regarding the perceptions of management methods. However, similarities across groups that also align with scientific evidence may help to pinpoint strategies that are more likely to be successful.

4. Develop a conservation strategy to promote a sustainable Nassau grouper fishery in The Bahamas. (Chapter VII and Appendix I)

Chapter VI adopts an applied research approach to strengthen management strategies for fishery resources. The review synthesizes ecological information and available fisheries data for the most important contemporary and emerging coastal fisheries in The Bahamas to identify knowledge gaps and deficiencies in existing fisheries management. Case study species including Nassau grouper have been selected to examine the efficacy of current management practices, and provide recommendations for enhancing conservation and management policies within the country. This is followed by a general discussion (Chapter VII), synthesising the main findings from the thesis, while acknowledging the associated limitations of the approaches used. A species management plan was developed (Appendix I) and research directions were also outlined to expand upon the portfolio of information required for Nassau grouper.

Chapter I

Integrating population biology into conservation management for endangered Nassau grouper *Epinephelus striatus*

Manuscript published in Marine Ecology Progress Series

Authors: Sherman KD, Dahlgren CP, Stevens JR, Tyler CR

Vol. 554: 263–280, 2016
doi: 10.3354/meps11771

MARINE ECOLOGY PROGRESS SERIES
Mar Ecol Prog Ser

Published July 28

REVIEW

Integrating population biology into conservation management for endangered Nassau grouper *Epinephelus striatus*

Krista D. Sherman[1,2], Craig P. Dahlgren[2], Jamie R. Stevens[1], Charles R. Tyler[1,*]

[1]Biosciences, Geoffrey Pope Building, University of Exeter, Stocker Road, Exeter, Devon EX4 4QD, UK
[2]Science and Policy, Bahamas National Trust (BNT), PO Box N-4105, Nassau, Bahamas

ABSTRACT: Groupers are a phylogenetically diverse group and include many ecologically and economically valuable predatory marine fishes that have experienced drastic population declines. Reproduction via spawning aggregations increases the vulnerability of grouper species such as Nassau grouper *Epinephelus striatus* to overfishing, and this is likely to be a major contributing factor to population declines. However, the lack of information pertaining to population structure and dynamics of Nassau grouper spawning aggregations has impeded effective ecosystem-based fisheries management for remaining stocks. Worldwide, The Bahamas has the largest number of known Nassau grouper spawning aggregations, yet very little is known about the overall status of groupers in the region. Landings of Nassau grouper in The Bahamas have declined by 86% in the last 20 years from a peak of 514 t in 1997. Available data suggest that existing management measures are failing in their attempts to prevent further declines. Effective management strategies are urgently needed that balance ecological and socioeconomic considerations to enable a sustainable Nassau grouper fishery. This review provides an analysis of the reproductive and population biology of Nassau grouper and a suggested framework to direct future research efforts for enhancing conservation management of this endangered marine fish species.

KEY WORDS: Fisheries management · Population structure · Marine protected area · MPA · Spawning aggregation · Genetic diversity · Microsatellite · Single nucleotide polymorphisms

INTRODUCTION

Spawning aggregations—where conspecific fish come together in high densities to release their gametes (eggs and sperm) into the water or onto a suitable substrate—occur in over 100 reef fish species (Sadovy de Mitcheson & Colin 2012, Russell et al. 2014), many of which have economic importance, including parrotfish (Scaridae), groupers (Epinephelidae) and snappers (Lutjanidae) (Heemstra & Randall 1993, Beets & Hixon 1994, Colin 1996, Morris et al. 2000, Sadovy de Mitcheson et al. 2008, Coleman et al. 2010, Craig et al. 2011, Choat 2012, Colin 2012). The existence of these spawning aggregations is threatened by a range of environmental and anthropogenic pressures, including overfishing (Sadovy de Mitcheson & Erisman 2012, Robinson et al. 2014), habitat loss and degradation (Robinson & Samoilys 2013), invasive species (Muñoz et al. 2011) and climate change (Cheung et al. 2012). According to a recent report, 26% of aggregating marine fish species have decreased, 4% have disappeared and the status of most other aggregating fish populations are unknown (Russell et al. 2014).

Groupers belonging to the family Epinephelidae are a phylogenetically diverse group of predatory fishes comprising 6 subfamilies, 12 genera and 163 species that inhabit a wide range of marine habitats

*Corresponding author: C.R.Tyler@exeter.ac.uk

Publisher: Inter-Research · www.int-res.com

(Baldwin & Johnson 1993, Heemstra & Randall 1993, Craig & Hastings 2007, Craig et al. 2011, Schoelinck et al. 2014). Fishes from the genus *Epinephelus* are paraphyletic, with larger bodied species sharing a common ancestry despite a geographic discontinuity between the Atlantic and Eastern Pacific (Craig & Hastings 2007). Groupers from the subfamily Epinephelinae are found throughout subtropical and tropical oceans and account for a substantial percentage (~12%) of annual global fisheries revenue, particularly in Asia via the live fish trade and aquaculture, and in developing Caribbean countries via commercial, recreational and subsistence fisheries (Buchan 2000, Rudd & Tupper 2002, Craig et al. 2011, Sadovy de Mitcheson et al. 2013, FAO 2014, Western Central Atlantic Fishery Commission 2014). In Asia, a consistently high demand for groupers has driven prices up to US$100 kg^{-1} (FAO 2014). Globally, grouper stocks have declined by around 60% over the last 3 decades (Sadovy de Mitcheson et al. 2008, Sadovy de Mitcheson & Colin 2012), with some even greater regional and local population declines (Cheung et al. 2013, C. P. Dahlgren et al. unpubl. data). These declines are believed to be due to a combination of grouper life history characteristics and unsustainable fishing practices. Many of the larger grouper species are *K*-selected strategists with long lifespans, slow growth rates and delayed sexual maturity (Coleman et al. 1996, 2000, Sadovy de Mitcheson & Colin 2012). For many grouper species, much of the exploitation occurs at spawning aggregation sites, which increases their susceptibility to over-exploitation (Heemstra & Randall 1993, Musick 1999, Coleman et al. 2000, Morris et al. 2000, Sadovy de Mitcheson et al. 2013). Overfishing of grouper may lead to alterations in the structure of spawning aggregations (Carter et al. 1991, Sadovy & Domeier 2005, Sadovy de Mitcheson & Colin 2012), and this in turn may impact negatively on long-term reproductive success.

Recognition of the vulnerability of grouper stocks has led to conservation efforts to evaluate the status of populations to help prevent further declines at both regional and global scales. Of the grouper species assessed to date, the International Union for the Conservation of Nature (IUCN) Grouper and Wrasses Specialist Group has classified 13% as threatened (i.e. critically endangered, endangered or vulnerable) and 14% as near threatened. For approximately 30%, there are insufficient data to make any valued judgement on their status (IUCN 2015). The lack of species-specific information pertaining to population structure and dynamics of spawning aggregations is one of the major impediments for effective ecosystem-based fisheries management for remaining grouper stocks.

Nassau grouper *Epinephelus striatus* (Bloch 1792), one of the most important grouper species economically, form annual transient fish spawning aggregations (FSAs; Domeier 2012). This is when a group of conspecific fish gathers at a site located at a considerable distance outside their home range for the purpose of spawning. At these sites, fish densities are greater than those outside of the aggregation area and often represent the total reproductive effort for participating individuals (Domeier 2012). Up to 100 000 Nassau grouper have been observed in a single spawning aggregation off Cat Cay, Bimini in The Bahamas (Smith 1972).

Sixty to eighty Nassau grouper spawning aggregation sites have been identified globally (Sadovy & Eklund 1999), but many of these have been lost due to overfishing (Sadovy de Mitcheson et al. 2008). The Bahamas has the largest number (ca. 30 sites) of known viable (reproductively active) Nassau grouper spawning aggregations (Sadovy & Eklund 1999, Cheung et al. 2013; Fig. 1), but with significant declines apparent at many of the historic aggregation sites (e.g. Cat Cay, Bimini and High Cay, Andros). However, sighting frequencies and densities of Nassau grouper are still 2 to 3 times higher in The Bahamas relative to other parts of the Caribbean (Stallings 2009, Dahlgren et al. 2016). For this region in particular, there is an urgent need to better understand the reproductive biology and evaluate the status of remaining grouper stocks to strengthen and inform national and regional management strategies. Cheung et al. (2013) reported that local fishers have recognised decreases in abundance and sizes of Nassau grouper caught at spawning aggregation sites since the 1990s, and the majority (82%) were concerned about the long-term viability of the fishery. If Nassau grouper populations continue to be overfished at their current rate (Cheung et al. 2013), this will likely result in the collapse of the fishery within the next few decades, with very considerable economic and social ramifications for The Bahamas and the wider Caribbean. As a highly prized commercial fish species, the Nassau grouper is very important to the livelihoods of thousands of fishermen in The Bahamas (Buchan 2000, Cushion & Sullivan-Sealey 2008). It is imperative, therefore, that management plans balance ecological and socioeconomic considerations to ensure a sustainable Nassau grouper fishery. This review provides an analysis of the reproductive and population biology of Nassau grouper with

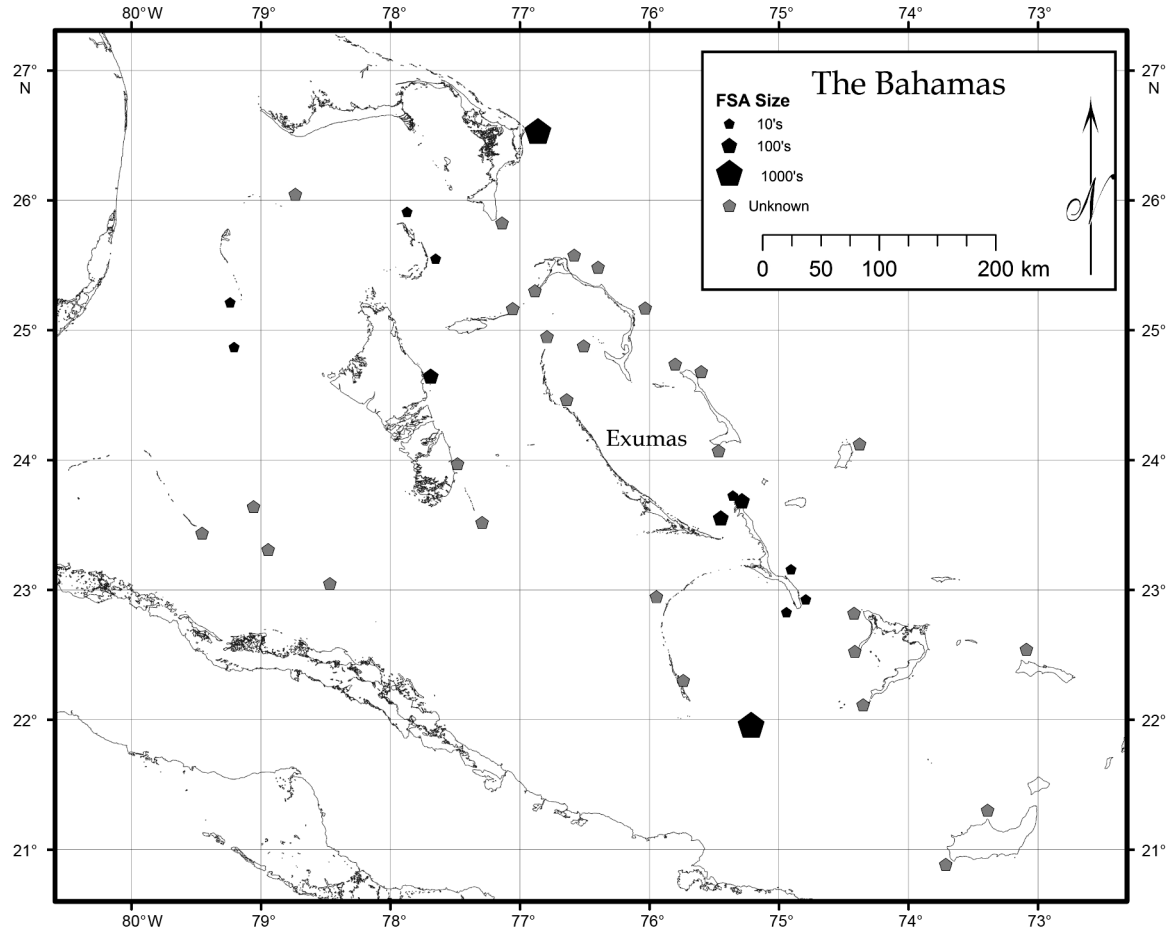


Fig. 1. Approximate locations of known and anecdotal Nassau grouper *Epinephelus striatus* fish spawning aggregations (FSAs) in the Bahamian archipelago. Spawning aggregation sites are denoted by black pentagons of various sizes corresponding to estimates of Nassau grouper abundance: small = 10's of fish, medium = 100's of fish, large = 1000's of fish. Grey pentagons = unknown (i.e. no *in situ* data or unverified spawning aggregation site). Figure based on the most recent data available from each site

a view to help support and direct future research efforts and approaches for enhancing conservation management of this endangered marine fish species.

BASIC BIOLOGY AND ECOLOGY

Nassau grouper, subfamily Epinephelinae, are slow growing, relatively long-lived (ca. 30 yr) predatory fish growing up to a maximum weight of 27 kg (Albins et al. 2009, Froese & Pauly 2014) that inhabit nearshore habitats up to 255 m in depth throughout the tropical Western Atlantic, Caribbean Sea and parts of the Gulf of Mexico (Starr et al. 2007, Albins et al. 2009, Froese & Pauly 2014). Normally Nassau grouper are light grey or olive to reddish brown in colour, with 5 distinct dark bars along the body, but they may undergo rapid colour and pattern changes that are associated with various behaviours (Archer et al. 2012, Watson et al. 2014). Nassau grouper exhibit dietary shifts during ontogeny. As larvae and pelagic juveniles, they consume primarily zooplankton and copepods (Grover et al. 1998); as demersal juveniles in nearshore benthic habitats (i.e. mangroves, seagrasses and macroalgal clumps), they feed mainly on crustaceans (Eggleston 1995, Eggleston et al. 1998, Grover et al. 1998, Dahlgren & Eggleston 2001, Dahlgren et al. 2006); as late juveniles or subadults living on hard bottom and patch reefs, they consume both invertebrates and fish (Dahlgren & Eggleston 2000, Camp et al. 2013); and as adults, living in deeper and more rugose reef habitats, they predominantly feed on fish (Eggleston et al. 1998). The timing of these ontogenetic shifts is mediated by a combination of predator–prey dynamics, ecophysiological processes and habitat suitability (Dahlgren & Eggleston 2001, Young et al. 2006, Semmens et al. 2008, Stallings 2008, Camp et al. 2013).

Major loss of any of the habitats utilised by groupers during ontogeny is likely to influence their abundance and survival. Perhaps the greatest habitat losses influencing grouper populations arise from alterations or destruction of nearshore nursery areas, including mangroves and seagrasses, and by the declining state of coral reef systems (Dahlgren & Eggleston 2001, Gardner et al. 2003, Lotze et al. 2006, Semmens et al. 2008). Ellis et al. (1997) demonstrated that changes in water temperature experienced by early life stages of Nassau grouper may have profound effects on their food consumption, development and growth rate, and these factors may play a significant role in the annual fluctuations in the survival of this species. This may be compounded by the fact that these early life stages already experience high mortality rates due primarily to predation (Choat 2012) and disease susceptibility (Harikrishnan et al. 2011). Sea temperature may also affect adult grouper directly by affecting metabolism, reproduction, growth and behaviour (Colin 1992, Watanabe et al. 1995b). Other environmental stressors that are likely to affect grouper survival directly include predicted climate-related changes in ocean chemistry (e.g. ocean acidification) (Young et al. 2006, Semmens et al. 2008, Cheung et al. 2012, Sunday et al. 2014). However, to date, very little research has been done examining the impacts of environmental stressors on the different life stages of Nassau grouper.

Adult Nassau groupers are mesopredators (trophic level = 4.1; Froese & Pauly 2014) and play a vital role in maintaining the balance of coral reef ecosystems (Eggleston et al. 1998, Huntsman et al. 1999, Mumby et al. 2006, Stallings 2008, Mumby et al. 2012). They also form symbiotic relationships with cleaner species (e.g. Gobidae and Labridae) and are often observed at coral reef cleaning stations (Sluka et al. 1993, Sadovy & Eklund 1999). It has been estimated, using a combination of bioenergetic and experimental models, that aggregating groupers contribute significantly to nutrient supply, at 1.54 to 4.61 g m^{-2} and 0.75 to 2.27 g m^{-2} of nitrogen and phosphorus, respectively (Archer et al. 2015). Nassau grouper, therefore, play fundamental roles not only in trophic dynamics (as predators and prey) but also in wider aspects of ecosystem function and reef resilience.

REPRODUCTIVE BIOLOGY

Reproductive strategies of fish vary widely and have evolved to optimally provide for their offspring while balancing the ecophysiological costs to the adults (Murua & Saborido-Rey 2003, Molloy et al. 2012). The reproductive potential of a species is dependent on several factors, including age and size at maturation (which generally correlate positively with gamete quality and fecundity), sex ratios, size and condition of breeding adults, as well as the timing and length of spawning events (Carter et al. 1991, Beldade et al. 2012, Choat 2012, Espigares et al. 2015).

The reproductive biology of Nassau grouper has been well studied (Shapiro 1987, Sadovy & Colin 1995, Watanabe et al. 1995b, Cushion et al. 2008, and references therein) due to its economic importance and the interest in the species for aquaculture. Nassau grouper are gonochoristic and reach sexual maturity between the ages of 4 and 8 yr (≥480 mm total length [TL]) (Sadovy & Colin 1995, Sadovy & Eklund 1999, Cushion et al. 2008, Froese & Pauly 2014). The species goes through a hermaphroditic stage as juveniles and adults are capable of sex change, although the drivers for sex change are not well established (Colin 1992, Watanabe et al. 1995a, Cushion et al. 2008). Sadovy & Colin (1995) identified environmental and social triggers as possible sex change drivers, but there has been no consensus on this and sex change in wild populations has not been documented.

Potential techniques for sex determination in wild fish populations that are not sexually dimorphic include the use of invasive (e.g. gonad removal; Cushion et al. 2008) and non-invasive (e.g. ultrasound; Whiteman et al. 2005) sampling techniques. Applying histology to determine gender and stage of sexual maturity requires terminal sampling, although techniques such as laparoscopy have also been employed to collect a small section of the gonad for staging from live fish (e.g. Falahatkar et al. 2011, Matsche et al. 2011). In some other fish species, DNA sex probes have been developed that can be applied non-destructively using a small fin clip or scale sample, e.g. for medaka (Matsuda et al. 1998), stickleback (Shikano et al. 2011), roach (Tyler & Jobling 2008) and salmon (Eisbrenner et al. 2014), but such a probe has not been developed for any grouper species. Development and utilisation of this technique for groupers seems to be logical given that fin clip samples are routinely collected for genetic analysis (Colin 2012). Additionally, analysis of previously collected fin clip samples could be compared with contemporary samples to report on any shifts in sex ratios that may have occurred and better understand the impacts of overexploitation on demographics and reproductive success.

Another approach to sex fish involves the measurement of blood vitellogenin — a precursor of egg yolk produced in female oviparous fish in response to oestrogens. Blood vitellogenin has been used as a non-destructive biomarker to effectively discriminate between males and females, even as juveniles, and to stage female ovary development. Heppell & Sullivan (1999) developed and successfully applied a vitellogenin-based enzyme-linked immunosorbent assay (VTG ELISA) to classify phases of sexual development in the grouper, *Mycteroperca microlepis*. The antibody for this assay has been shown to cross-react well with Nassau grouper VTG, allowing for its application to this species. Concentrations of VTG in blood plasma of female Nassau grouper have been measured at concentrations ranging between 0.02 and 4.66 mg ml^{-1} and are positively correlated with sexually maturity (Heppell & Sullivan 1999). Measurement of VTG in wild fish has the potential to better establish demographics (e.g. spawning stock biomass and sex ratios), variability in spawning seasonality and viability of remaining spawning aggregations, but has yet to be applied in this regard.

Very little has been established relating to the function of sex steroid hormones in the reproductive cycle of Nassau grouper. Nassau grouper, however, have been successfully induced to spawn in laboratory settings using various steroid hormones, including carp pituitary hormone, luteinizing hormone-releasing hormone and human chorionic gonadotropin (Tucker & Woodard 1991, Watanabe et al. 1995a). Captive fish have also been observed spawning in aquaria and aquaculture cages without the use of hormone injections (Watanabe et al. 1995a, Tucker 1999). Reproduction in wild populations is thought to be density dependent (Colin 1992, Sala et al. 2001, Aguilar-Perera 2006), but the minimum threshold required to sustain a spawning aggregation has not been determined. Colin (1992) observed spawning in an aggregation containing as few as 10 Nassau grouper, but noted that overall spawning behaviour was reduced when compared with larger aggregations observed at other spawning sites during that period. This suggests that heavily fished spawning aggregation sites may have a lower relative productivity for any given fish biomass and are thus more susceptible to collapse than at less-exploited sites. Preferably, non-lethal sampling approaches should be used to assess the reproductive status and demographics of endangered Nassau grouper.

Nassau grouper migrate up to 300 km to and from their resident reefs along the continental shelf to reproduce at spawning sites (Bolden 2000, C. P. Dahlgren et al. unpubl.). Migrating speeds and distances vary intraspecifically (Colin 1992, Bolden 2000, Starr et al. 2007, C. P. Dahlgren et al. unpubl.) and recent studies indicate that courtship-associated sounds play an integral role in facilitating this process. These auditory signals may also help first-time spawners to navigate to spawning sites (Schärer et al. 2012, Rowell et al. 2015). Analysis of movement patterns of tagged groupers suggest that females exhibit spawning site fidelity, often migrating past several spawning aggregations to reach their 'home' spawning site (Heppell et al. 2009, C. P. Dahlgren et al. unpubl). In the Cayman Islands and Belize, 50% or more individuals make multiple spawning migrations (Semmens et al. 2007, Starr et al. 2007), but in The Bahamas fish appear to generally make single migrations (C. P. Dahlgren et al. unpubl., K. L. Stump et al. unpubl.).

Available information on spawning sites has shown they are highly diverse, varying in size, habitat type and water depth, but are often in close proximity to drop-offs and continental shelf edges (Smith 1972, Colin 1992; Aguilar-Perera & Aguilar-Dávila 1996, Whaylen et al. 2004, Heyman & Kjerfve 2008, Kobara & Heyman 2008, Kobara et al. 2013). The behavioural and physiological triggers associated with the formation and structure of migrating groups to, from and within spawning sites and the timing of gamete release remain poorly understood.

Migration and spawning activities have been linked to abiotic factors, including lunar phase, water temperature, tides and currents (Colin 1992, Shenker et al. 1993, Takemura et al. 2010, Heppell et al. 2011). Colin (1992) suggested that the optimal spawning temperature for Nassau grouper is between 25 and 26°C, which may explain latitudinal variation observed in the timing of spawning events throughout the species' geographic range. Reproduction in Nassau grouper is highly synchronised with the full moon (Sadovy & Eklund 1999). In other fish species that use lunar phases in the timing of their spawning, expressions of clock genes (e.g. *rfPer2*) have been shown to change with moonlight intensity and may be instrumental in regulating reproductive cycles (Takemura et al. 2010). However, no studies have examined the functional role of clock genes or other regulatory genes associated with the timing of reproduction in Nassau grouper. Such data would offer additional insights into the molecular mechanisms driving reproduction and help to explain observed differences in spawning seasonality of the species.

Reproduction via spawning aggregations may optimise fertilisation success, maximise larval survivor-

ship, and increase genetic mixing (Colin 1992, Domeier 2012). Patterns of larval dispersal, behaviour and retention throughout the native range of Nassau grouper have not been extensively investigated (Colin 1992, 1995, Heppell et al. 2009). Larval development occurs over a 35 to 50 d period, with a mean larval dispersal duration of 42 d (Tucker & Woodard 1991, Colin et al. 1997), followed by recruitment to macroalgal habitats as juveniles (25–35 mm TL) and a transition to reef habitats after 10 to 12 mo (Dahlgren & Eggleston 2001). Larval behaviour is not well understood and recruitment events are highly variable (Grover 1993, Shenker et al. 1993, Colin 1995, Colin et al. 1997, Grover et al. 1998, Heppell et al. 2011). Colin (1995) demonstrated that mesoscale gyres in the Exuma Sound could passively transport Nassau grouper recruits to natal spawning sites. Similar studies tracking the direction of surface and subsurface currents off the Little Cayman spawning aggregation site have shown that eddies retain larval Nassau grouper near spawning areas, which the authors postulate as a mechanism for self-recruitment (Heppell et al. 2011).

Various models have been developed to identify patterns of larval distribution. They include biophysical models such as the Lagrangian stochastic model, a larval tracking model of the Connectivity Modelling System (Paris et al. 2013) and the random-walk model (Rivera et al. 2011). With appropriate ecological (e.g. tagging studies; C. P. Dahlgren et al. unpubl.) and genetic data (e.g. parentage analysis; Almany et al. 2013), these models might usefully be applied to Nassau grouper to elucidate connectivity, source–sink dynamics and recruitment variability over broad spatial scales. Better understanding of survivorship in early life stages of Nassau grouper would provide key insights into larval distribution pathways and recruitment stochasticity, which would in turn clarify patterns of population structure and connectivity in adult fish. Addressing knowledge gaps in relation to the underlying mechanisms that influence and/or control the reproductive development and biology of Nassau grouper throughout its complete life history are required to set sustainable fishery targets and inform conservation management.

CONSERVATION STATUS AND SOCIOECONOMICS

Conservation status

Given its declining status, the Nassau grouper is now classified as Endangered on the IUCN Red List (IUCN 2015) and threatened under the United States Endangered Species Act (Cornish & Eklund 2003, Albins et al. 2009). A variety of management measures have been implemented in an attempt to counteract the observed declines of Nassau grouper and to conserve the remaining populations in The Bahamas and throughout the Caribbean. Examples include size limits, partial or full seasonal closures, quotas, fishing bans, establishment of marine protected areas (MPAs) and FSA closures and FSA MPAs. The successes of these management strategies vary. For example, even after a complete moratorium on fishing for over 15 yr, the Nassau grouper fishery in Florida and Bermuda has still not recovered (Sadovy & Eklund 1999, Luckhurst 2001). In contrast, permanent fishing bans on Nassau grouper FSAs in the Cayman Islands and St. Thomas have been effective in assisting some of those populations to stabilise and recover from overfishing (Whaylen et al. 2004, 2007, Kadison et al. 2010, Heppell et al. 2012).

Nassau grouper fisheries management in The Bahamas

In the late 1980s, the Government of The Bahamas established a minimum 3 lb size limit (≥1.36 kg) for landed grouper and in 1998 a partial seasonal closure for Nassau grouper was imposed at the High Cay, Andros spawning aggregation site (under the Fisheries Resources (Jurisdiction and Conservation) Act CH.244-11, Part V; http://laws.bahamas.gov.bs/cms/en). The 3 lb size limit (≥480 mm TL) was based on size at first maturation data of Nassau grouper from the Caribbean (Sadovy & Eklund 1999). Because the resources required to consistently enforce all reported Nassau grouper FSAs in The Bahamas are inadequate, seasonal closures were implemented to prohibit the capture, sale and possession of Nassau grouper. As a consequence, all Nassau grouper are off-limits to fishing during part of the reproductive season. However, national seasonal closures to protect fish at other spawning sites were not established until 2004 (Table 1). The declaration of the closed season was left to the discretion of the Minister of Agriculture and Fisheries and this resulted in confusion and inconsistencies for many years (Table 1). During this period, the seasonal closure typically extended for 1 to 2 mo between December and February. Subsequent research has shown seasonal variation in Nassau grouper spawning, and they can reproduce earlier during the November full moon

Table 1. Management timeline for the Nassau grouper *Epinephelus striatus* fishery in The Bahamas

Year(s)	Management measure	Details
1958	Establishment of the first Marine Protected Area	Limitations placed on catching marine resources (including Nassau grouper) within the 456 km^2 Exuma Cays Land and Sea Park
1986	Exuma Cays Land and Sea Park designated as a no-take marine reserve	All marine resources within the park's boundaries are protected
1987–1988	Fisheries Act sets minimum size limit	Landed grouper must be ≥3 lb (≥1.36 kg)
1998–1999	First partial closure of known Nassau grouper spawning aggregation site	High Cay, Andros is closed during the November–February full moons
2000	Partial closures of additional Nassau grouper spawning aggregation sites	Known spawning aggregation sites in Long Island are closed to fishing during the December full moon
2004	First national seasonal closure for Nassau grouper	Nassau grouper fishing banned during December–February
2005–2014	National seasonal closures	Annual closures set by the Minister of Agriculture and Fisheries. The closed season during this period varied from 1 to 3 mo
2015	Permanent annual seasonal closure established	Fisheries Act amended to prohibit fishing Nassau grouper during the months of December–February, which coincide with peak reproduction in The Bahamas

(Cushion et al. 2008, C. P. Dahlgren et al. unpubl.), suggesting that the current seasonal closure is insufficient. In 2015, the Fisheries Act was amended to include an annual 3 mo seasonal closure of Nassau grouper during 1 December–28 February. Additionally, all species within the boundaries of no-take marine reserves, e.g. the Exuma Cays Land and Sea Park (ECLSP), are protected from extraction activities including fishing. Despite seasonal closures and size restrictions, fishery-dependent data show steady declines in Nassau grouper landings throughout The Bahamas (Fig. 2).

Commercial fisheries landings

Commercial fishery landings data for The Bahamas is collected by the Department of Marine Resources (DMR). Historically, most Nassau grouper were landed in The Bahamas during the spawning season (Gascoigne 2002). Prior to 1994, Nassau grouper data were combined with data from all other grouper species, so figures on commercial landings for individual grouper species are only available for the period between 1994 and 2014. During this 20-yr period, a total of 4716 tonnes (t) of Nassau grouper were landed in The Bahamas, averaging 236 t yr^{-1} (Fig. 2). Landings in The Bahamas declined to 70 t in 2012 from a peak of 514 t in 1997, with an overall decline of 86% over the past 2 decades (i.e. between 1994–2014) (Fig. 2). The DMR has acknowledged that commercial landings data in The Bahamas is often under-reported (see Cheung et al. 2013). Declines in Nassau grouper landings began before the implementation of the 2004 seasonal closure and have persisted for 20 yr. During the months of December-February, approximately 50 and 20% of Nassau grouper have been landed in The Bahamas

Fig. 2. Total commercial Nassau grouper *Epinephelus striatus* landings (weight and value) for The Bahamas during 1994–2014 as reported to The Bahamas Department of Marine Resources

before and after the implementation of the closed season. If the entire reproductive season (November–March) for the species is examined, 61 and 41% of fish have been landed in the country before and after seasonal closures. Available catch per unit effort (CPUE) data indicate declines over time (see Cheung et al. 2013). While the observed declines in CPUE may be due to a shift to other targeted species, the overall trend, coupled with the 20 yr decline in landings and the proportion of fish still harvested during the closed season, provides strong evidence to support that the Nassau grouper fishery is in serious decline. This is of major concern for The Bahamas, given that annual economic contributions from the fishery range between US$ 620 thousand and 2.8 million, with an average of US$1.5 million yr^{-1} (Fig. 2). It is also important to mention that these figures provide conservative estimates of the total fishery landings (see Cheung et al. 2013) and exclude revenue from recreational and tourism-related activities (Rudd & Tupper 2002). Economic valuation studies for Nassau grouper are needed (Rudd & Tupper 2002) and would be useful to help explain the socioeconomic benefits of conserving Nassau grouper to a broad range of stakeholders.

POPULATION STRUCTURE AND DYNAMICS

The Bahamas contains the largest proportion of global Nassau grouper spawning aggregation sites, with up to 30 located throughout the archipelago (Sadovy & Eklund 1999, Cheung et al. 2013, Kobara et al. 2013; Fig. 1). FSA research has been inconsistent and has focused on only a few sites from 3 islands: Bimini (Smith 1972, Gascoigne 2002), Andros (Ray 2000, Ehrhardt & Deleveaux 2007) and Long Island (Colin 1992). In contrast, consistent monitoring has been conducted at Nassau grouper FSAs in the Cayman Islands over a period of 10 yr (e.g. Whaylen et al. 2007) and in Puerto Rico for 11 yr (e.g. Schärer et al. 2010, Schärer-Umpierre et al. 2014). Vo et al. (2014) recently completed a stock assessment in the Turks and Caicos Islands where there are no management measures for Nassau grouper and have indicated that the population is relatively healthy (biomass of 0.58 t km^{-2} in 2008) compared with other parts of the Caribbean.

Attempts to evaluate the status of the Bahamian Nassau grouper fishery have relied heavily on fishery landings data, anecdotal information from fishers and modelling (Gascoigne 2002, Ehrhardt & Deleveaux 2007, Cheung et al. 2013). As an example, a previous estimate on Nassau grouper stocks by Ehrhardt & Deleveaux (2007) was based on a single historical spawning aggregation site off High Cay, Andros. Their findings lacked *in situ* population data and were based on fishery landings and hydroacoustic assessments of fish abundances. Furthermore, the hydroacoustic assessments were not validated and there was a wide discrepancy in these data compared with diver observations of Nassau grouper at that site during the same period (Ray 2000). Divers reported abundances of fish at High Cay to be only 4 to 5% of hydroacoustic assessments (Ray 2000). The most recent studies from The Bahamas indicate abundances of Nassau grouper have declined over the past 2 decades between 70 and 90% in several historical locations (e.g. High Cay), and only 2 of 6 documented spawning aggregation sites in Long Island are still active (C. P. Dahlgren et al. unpubl. data). Visual observations estimate fish abundance at extant Long Island sites to be between only 200 and 700 grouper, far less than the thousands of fish reported in the past at these spawning aggregations (Colin 1992), although Colin (1992) did report that some of the FSAs in Long Island were fished out over the course of 1 to 2 spawning seasons. Similar observations of the rapid decline in abundance of Nassau grouper FSAs have also been reported from the Caribbean (e.g. Sala et al. 2001, Aguilar-Perera 2006). At present, the remaining stocks in The Bahamas experience high rates of poaching from local fishers, with up to 25 to 35% of fish removed from spawning aggregation sites annually (C. P. Dahlgren et al. unpubl. data). In contrast with other countries that have fewer spawning sites (e.g. Heyman & Requeña 2002, Heppell et al. 2012), very few reported Bahamian spawning aggregations have been validated through scientific methods (e.g. *in situ* diver surveys, telemetry, hydroacoustic assessments). Indeed, whether or not many of these locations still support active aggregations is essentially unknown. This is due in part to the logistical difficulties and financial costs associated with conducting fieldwork, especially in the remote areas where spawning sites often occur, and the lack of local capacity to collect data from widely dispersed spawning aggregation sites throughout the Bahamian archipelago. Population genetic approaches offer the potential to better establish population structure and dynamics over such an expansive area (~233 000 km^2 of sea) and the fin clips or tissue samples required for these approaches could be collected with relative ease from live or recently killed specimens.

Overfishing is commonly cited as the primary cause for local collapses and global population

declines of Nassau grouper (Colin 1996, Aguilar-Perera 2006, Sadovy de Mitcheson et al. 2008, Albins et al. 2009, Cheung et al. 2013). Typically, larger individual groupers are removed from the population before they reproduce, which means that stocks are not being replenished. Removing the majority of large, highly fecund groupers over time is likely to reduce reproductive success and impact further on population sustainability (Heppell & Sullivan 1999, Sadovy & Domeier 2005) via decreasing genetic variability and thus population adaptability.

The use of molecular techniques to investigate genetic diversity, population structure and connectivity in marine ecosystems has increased substantially over the last 2 decades (Shulman & Bermingham 1995, Palumbi 2004, Cowen et al. 2006, Craig & Hastings 2007, Harrison et al. 2012, Beldade et al. 2014, Jackson et al. 2015). Molecular methods are now being applied in the assessments of population structure and genetic diversity (Silva-Oliveira et al. 2008), investigating evolutionary processes and local adaptation to natural or anthropogenic stressors (Paris et al. 2015), exploring genes controlling or regulating diseases (Teng et al. 2008), understanding sexual development (Luo et al. 2010), informing conservation management plans (Reiss et al. 2009) and in predicting the impacts of climate change (Nielsen et al. 2009, Davey et al. 2011, Horreo et al. 2011, Narum et al. 2013, Hemmer-Hansen et al. 2014). However, use of these approaches to understand the breeding biology and population dynamics of groupers is still in its infancy. Population structure in marine fish is influenced by interacting biological, ecological, environmental and anthropogenic processes and vary over spatial and temporal scales (see reviews by Nielsen et al. 2009, Reiss et al. 2009, Takemura et al. 2010, Hemmer-Hansen et al. 2014). Barriers to gene flow appear to be more subtle in the ocean, where larvae are dispersed with ocean currents and fish can migrate over vast distances (Lindeman et al. 2000, Palumbi 2004). This makes it challenging to assess population structure, dynamics and genetic differentiation in marine fish.

Polymorphic markers, such as DNA microsatellites or simple sequence repeats (SSR) and single nucleotide polymorphisms (SNPs), are now commonly used to more accurately assess genetic population structure and diversity among wild fish populations (Sunnucks 2000, Hutchinson et al. 2001, Zatcoff et al. 2004, Hauser & Carvalho 2008, Griffiths et al. 2010, Hohenlohe et al. 2010, Wang et al. 2013, Paris et al. 2015). Although evidence of population structuring has been documented in coral reef fish (e.g. Shulman & Bermingham 1995, Bay et al. 2008), only a few studies have assessed population structure in Epinephelinae groupers (Rivera et al. 2004, 2011, Zatcoff et al. 2004, Maggio et al. 2006, Silva-Oliveira et al. 2008, Beldade et al. 2014, Jackson et al. 2014, 2015) and there is a paucity of information regarding genotypic variation within and among spawning aggregations generally.

Using polymorphic DNA microsatellite loci, Zatcoff et al. (2004) found populations of red grouper *E. morio*, the most closely related species to Nassau grouper, and scamp *Mycteroperca phenax*, another aggregating marine fish, to be genetically similar. Both species are heavily fished, but no discernable effects on genetic diversity and population structure were detected from samples across the Atlantic and Gulf of Mexico (Zatcoff et al. 2004). Silva-Oliveira et al. (2008) also found no evidence of subpopulation structure in goliath grouper *E. itajara* from Brazil. In contrast, restriction fragment length polymorphism of NADH dehydrogenase (ND2) revealed that Atlantic and Mediterranean dusky grouper *E. marginatus* populations were genetically distinct (Maggio et al. 2006). Cytochrome sequence analysis provided further evidence for genetic variation and a growing population within the Mediterranean (Maggio et al. 2006). Similarly, Rivera et al. (2011) also found distinct populations of Hawaiian grouper *E. quernus*. Comparisons of microsatellite and mitochondrial DNA (mtDNA) data with pelagic larval dispersal models were used to demonstrate high connectivity between Hawaiian grouper populations and to identify important source–sink areas. This information was used to recommend protection of Hawaiian groupers in the mid-archipelago, an area where the highest levels of genetic diversity were observed (Rivera et al. 2011). Jackson et al. (2015) used a similar approach for leopard grouper *M. rosacea* in the Gulf of California to inform marine reserve design that would protect areas with the greatest genetic diversity and support connectivity between subpopulations.

Information on the genetic population structure and dynamics of Nassau grouper populations is scarce (Hateley 1995, Stevenson et al. 1998, Jackson et al. 2014; Table 2). Earlier assessments on Nassau grouper populations using enzyme electrophoresis and mtDNA showed low to moderate genetic variation, suggesting the existence of a single population comprised of randomly mating individuals in the northern Caribbean (Hateley 1995). Given that statistical measures used in population genetics (e.g. heterozygosity and F_{ST}) are based on frequencies of

Table 2. Chronological summary of available genetic data on Nassau grouper. The number of microsatellite loci used in each study is reported in square brackets

Year	Location (no. of sites)	No. of DNA samples	Techniques used	Purpose	Source
1998	Belize (4) USA (1) Cuba (1) The Bahamas (1)	396 97 21 23	Microsatellites [2]	Population structure	Stevenson et al. (1998)
2005	Cayman Islands (2) Turks and Caicos (1) Belize (1) The Bahamas (1)	Total: 264	Protein electrophoresis [20]	Population structure	Hateley (1995)
2012	Belize (1) Cayman Islands (1) US Virgin Islands (1)	50 50 50	Microsatellites [10]	Microsatellite primer development	Abelló et al. (2012) and Jackson et al. (2012)
2012	Cayman Islands (2) US Virgin Islands (1)	20 20	Microsatellites [15]	Microsatellite primer development	Bernard et al. (2012)
2014	Mexico (1) Belize (4) Cuba (2) Cayman Islands (3) USA (1) The Bahamas (3) Turks and Caicos (1) Puerto Rico (1) US Virgin Islands (1) British Virgin Islands (1) Antigua (1)	Total: 620	Mitochondrial DNA, microsatellites [9] and single nucleotide polymorphisms	Population structure	Jackson et al. (2014)

alleles, highly polymorphic microsatellite loci spread throughout the genome considerably increase the resolution and replication required to differentiate between populations when compared with maternally inherited mtDNA (see reviews by Sunnucks 2000 and Selkoe & Toonen 2006). More recently, Jackson et al. (2014) used a suite of molecular markers, including microsatellites, mtDNA and SNPs, to investigate genetic connectivity and variability of Nassau grouper populations throughout the Caribbean and The Bahamas. They found considerable regional variation in the genetic composition of Nassau grouper with evidence of subpopulations and suggested that these differences may be attributed to oceanographic variability, which restricts larval dispersal (Jackson et al. 2014).

Spawning migrations observed through the tagging of Nassau grouper in the central Bahamas are an order of magnitude greater in distance compared with other parts of the Caribbean region (C. P. Dahlgren et al. unpubl.) and appear to be consistent with genetic evidence of population structure in The Bahamas. Differences in the elemental composition of otoliths from fish from The Bahamas and Belize also provide evidence in support of regional and within-country population substructuring (Patterson et al. 1999). Bahamian populations of Nassau grouper may be self-sustaining and genetically distinct compared with other populations (Patterson et al. 1999, Cowen et al. 2006, Jackson et al. 2014). Yet, the genetic diversity, structure and effective population sizes (N_e) of Nassau grouper populations remain poorly understood and unquantified for most of The Bahamas. Currently, microsatellites and SNPs appear to provide the best resolution for understanding the genetic architecture and current demographics of these fish and detecting whether any recent changes have occurred to their population structure (Jackson et al. 2014).

Previous research has shown that reduced genetic diversity and variation in wild fish populations is often linked to drastic declines in effective population size, which can occur because of overfishing or historical bottlenecks (Smith et al. 1991). Understanding whether Nassau grouper populations in The Bahamas are comprised of single or multiple populations as well as the intraspecific differences and current demographics (i.e. abundance, age, sex ratios and size) of remaining populations is key to developing a robust conservation management strategy for

the fishery. Although Nassau grouper aquaculture may be promising, it is unclear how successful restocking initiatives would be without knowledge of genetic diversity and population structure of wild stocks (Roberts et al. 1995, Benetti 2014), which have adapted to survive and reproduce under environmental conditions that vary spatially.

Future research should be directed to expand knowledge regarding spatiotemporal patterns of genetic connectivity, population structure, differentiation, larval dispersal and recruitment using high-resolution genome-wide molecular markers, such as microsatellites and SNPs. These assessments will be greatly improved by using recently developed species-specific microsatellite primers for Nassau grouper (Bernard et al. 2012, Jackson et al. 2012) that have already been used in population genetic studies in parts of the Caribbean (e.g. Jackson et al. 2014).

ADDRESSING FUTURE RESEARCH NEEDS

The efficacy of various management measures for conserving the species is under evaluation (C. P. Dahlgren et al. unpubl. data), but fishery-dependent and independent data suggest inadequacies in the current approaches. For example, the size limit of fish landed needs to be amended from ≥3 lb (≥1.36 kg) to ≥5 lb (≥2.27 kg) to ensure that the species is allowed to reproduce at least once prior to capture. Although Nassau grouper may attain sexual maturity from ~3 lb (≥480 mm TL), first time migrators in The Bahamas are 540 mm TL or greater (C. P. Dahlgren et al. unpubl.). This differs from observations in the Caribbean, where size at first migration is ≥440 mm TL (e.g. Semmens et al. 2007). An improved data collection system is needed to more accurately capture landings data for the country to include sizes of fish, capture sites, dates, etc. Such information is important to understand national trends in abundance, size distribution of harvested specimens and CPUE.

Of the threats known to negatively impact Nassau grouper, overfishing—most notably at aggregation areas—is particularly detrimental. Failure to stem the decline in populations of Nassau grouper in The Bahamas is likely due to a number of factors including: (1) high market demand and non-compliance with established fishery regulations; (2) inadequate enforcement; and (3) limited understanding of reproductive biology, population structure and dynamics to inform fishery regulations. While increased funding may help to tackle issues relating to the capacity to enforce regulations and increase education and outreach efforts, from an ecological or biological perspective, the following questions need to be addressed to assist with improving conservation management for the species:

(1) How genetically diverse and connected are Nassau grouper populations within The Bahamas?

(2) What are the demographics, spawning stock sizes and predominant migration patterns of remaining Nassau grouper FSAs?

(3) What are the impacts of overfishing on the reproductive potential or success of the species?

(4) How should future no-take MPAs be designed to account for genetic connectivity and promote population recovery?

Spawning seasonality and migration patterns

Smith (1972) hypothesized that Nassau grouper migrate along continental shelf edges to FSAs. External tagging for mark-recapture studies and acoustic telemetry (e.g. Bolden 2000, Sala et al. 2001, Semmens et al. 2007, Starr et al. 2007), which involves the surgical implantation of acoustic tags to track fish movement, has proven useful in elucidating spatiotemporal patterns of Nassau grouper migrations. Investigations of Nassau grouper migratory behaviour have shown intraspecific differences with respect to movements both within home ranges (0.1–0.2 km) and along migratory pathways during the spawning season, where fish travel distances of 25 to >300 km (Carter et al. 1991, Colin 1992, Bolden 2000, Aguilar-Perera 2006, Semmens et al. 2007, Starr et al. 2007, Stevens-McGeever et al. 2013, C. P. Dahlgren et al. unpubl., K. L. Stump et al. unpubl.). However, in The Bahamas, migratory corridors, demographics (i.e. sex composition, size and age structure) of aggregating fish, and their origins remain largely unknown, and evidence suggests that they may be different from other parts of the Caribbean, and even within the country (C. P. Dahlgren et al. unpubl., K. L. Stump et al. unpubl.). Tagging and acoustic telemetry can be used to determine the origins of aggregators, reveal potential locations of FSAs, migratory pathways, migration frequency, FSA site fidelity, and connectivity between home reefs and Nassau grouper FSAs over various spatial scales (e.g. Bolden 2000, Semmens et al. 2007, Starr et al. 2007, Heppell et al. 2009, C. P. Dahlgren et al. unpubl., K. L. Stump et al. unpubl.). This type of information is critical to understand patterns of connectivity, inform marine spatial planning and prioritize enforcement efforts for active FSAs.

Genetic diversity and population connectivity

An important component of biodiversity conservation is genetic diversity (Moritz 2002). Yet, the extent to which gene flow is exchanged and migratory pathways occur among Nassau grouper FSAs and home reefs, in addition to source–sink dynamics of the species throughout the country's protected area system, is unknown. This is because little attention has been paid to migration patterns of adult fish species and the links between larval connectivity and genetic population structuring in the spatial design of existing MPAs in The Bahamas. Instead MPAs have been established based on opportunism and basic principles of marine reserve design, and socioeconomic, cultural and political considerations (Ray 1998, Moultrie 2012). Some Bahamian MPAs were created to conserve ecologically and economically valuable species and diverse marine habitats and to replenish important fishery species (e.g. Nassau grouper) through spillover effects (Sluka et al. 1996, Brumbaugh 2014). Indeed, several of the habitats required by Nassau grouper, including nearshore areas and deeper coral reef systems, are protected within parts of The Bahamas National Protected Area System (BNPAS).

For example, the oldest of the country's MPAs, the ECLSP, has some of the highest densities of Nassau grouper in The Bahamas and in the Caribbean region (Sluka et al. 1996, 1997, Dahlgren 2004, Stallings 2009, Dahlgren et al. 2016). This 456 km^2 no-take marine reserve encompasses both terrestrial and marine habitats, including seagrasses, mangroves, hard bottom areas, patch, fringing and forereefs (Sherman et al. 2013), supporting diverse fish and benthic communities. However, studies by Bolden (2000) and C. P. Dahlgren et al. (unpubl.) show that Nassau grouper migrate outside the ECLSP to spawn. These findings underscore the critical importance of integrating knowledge of reproductive biology and population dynamics into marine spatial planning. More recently, principles of MPA design have been refined to integrate connectivity at ecologically relevant scales (Botsford et al. 2009, White 2015). The Bahamas is working towards incorporating genetic structure and population connectivity into its MPA network for Caribbean spiny lobster *Panulirus argus*, staghorn coral *Acropora cervicornis*, elkhorn coral *Acropora palmata* and Nassau grouper through coupled biophysical larval dispersal models and genetic analysis (Kough et al. 2013, C. Paris pers. comm., K. D. Sherman et al. unpubl. data).

The Bahamas has entered into an agreement to implement the Program of Work on Protected Areas and, as part of the United Nations Convention on Biological Diversity and Caribbean Challenge Initiative, declared to protect 20% of its nearshore marine habitats by 2020 (Moultrie 2012). The designation of future MPAs that are to become a part of the BNPAS should not only be based on core principles of marine reserve design, but also take into account genetic diversity, population structure and connectivity, along with socioeconomic and cultural considerations. This type of MPA network would likely increase ecological and socioeconomic benefits to the country's populace and promote ecosystem resiliency.

Monitoring and stock assessments of FSAs

Available data suggest that spawning aggregations evolved to provide a suite of biological and ecological advantages to promote the survival of both adults and offspring by optimizing reproductive success and mediating interacting effects of predators and prey (Nielsen et al. 2009, Choat 2012, Domeier 2012, Molloy et al. 2012). However, very few studies have tested this and the evolutionary mechanisms associated with spawning aggregations remain unclear (Choat 2012), representing an important area for future research. Consistent monitoring of the status of Nassau grouper in home reefs and nearshore nursery habitats should be conducted to investigate trends or habitat-associated shifts in abundance and size distribution. Similarly, continued monitoring of Nassau grouper FSAs is also required to understand temporal variability in spawning behaviour and dynamics, and document recovery and/or declines of spawning stock biomass. Traditional ecological monitoring during the spawning and non-spawning periods will provide a better understanding of the health and population structure of Nassau grouper, which is fundamental to creating appropriate stock-recruitment management models. Such a model does not exist for The Bahamas and its development will help to ensure that sustainable exploitation rates are set to maintain a healthy fishery and the livelihoods of local fishers.

ADDRESSING BARRIERS TO IMPLEMENTATION

Although precautionary approaches (e.g. marine reserves) have been implemented to conserve Nassau grouper, these strategies have not necessarily

been effective at preventing further fishery declines (Fig. 2; Cheung et al. 2013, K. D. Sherman et al. unpubl. data). Marine protected area design, therefore, needs to include all aspects of a species' life history as well as spatial and temporal variability in population structure and dynamics, habitat connectivity, and spawning migration corridors, for optimal effectiveness (Chiappone et al. 2000, Grüss et al. 2014, Pittman et al. 2014, Rowell et al. 2015, C. P. Dahlgren et al. unpubl.).

The socioeconomic gaps that need to be addressed to improve compliance for fishery regulations are beyond the scope of this review. However, research has shown that recognising and assessing stakeholder perspectives is an important aspect of this process (Hilborn et al. 2005, Robinson et al. 2014, Wilson et al. 2016). These types of analyses are necessary to capture local knowledge, incentivise support, address misconceptions and change attitudes regarding the Nassau grouper fishery. Co-management approaches (e.g. Hilborn et al. 2005) should be integrated into management strategies to strengthen compliance for fisheries regulations and to reduce costs associated with enforcement. A combined approach, integrating tagging studies with genetic analysis, stock assessment modelling and FSA monitoring would yield insights into ecologically significant migratory corridors, reproductively successful and genetically diverse and/or distinct FSAs, and population structure of Nassau groupers throughout the archipelago. This information could be used to reveal areas where increased enforcement is warranted or new management measures need to be implemented. In doing so, the maximum benefits can be reaped from the limited resources available for monitoring and enforcement. Therefore, we strongly recommend a holistic approach that combines population genetics, acoustic telemetry, biophysical modelling and *in situ* ecological monitoring to provide a more rigorous and adaptive framework, to better inform conservation management strategies. This would vastly improve our understanding and lead to strengthened protection for remaining endangered Nassau grouper populations.

Acknowledgements. Mr Lindy Knowles, Science Officer for the Bahamas National Trust created the map of Nassau grouper FSAs. The Bahamas fishery data were provided to K.D.S by Michael Braynen and Lester Gittens of the Department of Marine Resources (Nassau, Bahamas). Financial support was provided to K.D.S. from the Shirley Oakes Butler (Overseas) and (Bahamian) Charitable Trust via the Lyford Cay Foundation and the East Nassau Rotary Club Foundation and to C.P.D. by the Atlantis Blue Project Foundation and a grant to the Perry Institute for Marine Science by the Disney Conservation Fund's Reverse The Decline Program. We have no conflicts of interest to declare. Constructive criticism of 4 anonymous reviewers greatly improved the manuscript.

LITERATURE CITED

Abelló P, Ai WM, Altmann C, Bernardi G and others (2012) Permanent genetic resources added to Molecular Ecology Resources Database 1 April 2012–31 May 2012. Mol Ecol Resour 12:972–974

Aguilar-Perera A (2006) Disappearance of a Nassau grouper spawning aggregation off the southern Mexican Caribbean coast. Mar Ecol Prog Ser 327:289–296

Aguilar-Perera A, Aguilar-Dávila W (1996) A spawning aggregation of Nassau grouper *Epinephelus striatus* (Pisces: Serranidae) in the Mexican Caribbean. Environ Biol Fishes 45:351–361

Albins MA, Hixon MA, Sadovy Y (2009) Threatened fishes of the world: *Epinephelus striatus* (Bloch, 1792) (Serranidae). Environ Biol Fishes 86:309–310

Almany GR, Hamilton RJ, Bode M, Matawai M and others (2013) Dispersal of grouper larvae drives local resource sharing in a coral reef fishery. Curr Biol 23:626–630

Archer SK, Heppell SA, Semmens BX, Pattengill-Semmens CV, Bush PG, McCoy CM, Johnson BC (2012) Patterns of color phase indicate spawn timing at a Nassau grouper *Epinephelus striatus* spawning aggregation. Curr Zool 58:73–83

Archer SK, Allgeier JE, Semmens BX, Heppell SA and others (2015) Hot moments in spawning aggregations: implications for ecosystem-scale nutrient cycling. Coral Reefs 34:19–23

Baldwin CC, Johnson GD (1993) Phylogeny of the Epinephelinae (Teleostei: Serranidae). Bull Mar Sci 52:240–283

Bay LK, Caley MJ, Crozier RH (2008) Meta-population structure in a coral reef fish demonstrated by genetic data on patterns of migration, extinction and re-colonisation. BMC Evol Biol 8:248

Beets J, Hixon MA (1994) Distribution, persistence, and growth of groupers (Pisces: Serranidae) on artificial and natural patch reefs in the Virgin Islands. Bull Mar Sci 55: 470–483

Beldade R, Holbrook SJ, Schmitt RJ, Planes S, Malone D, Bernardi G (2012) Larger female fish contribute disproportionately more to self-replenishment. Proc R Soc B 279:2116–2121

Beldade R, Jackson AM, Cudney-Bueno R, Raimondi PT, Bernardi G (2014) Genetic structure among spawning aggregations of the gulf coney *Hyporthodus acanthistius*. Mar Ecol Prog Ser 499:193–201

Benetti D (2014) New venture in Bahamas focuses on grouper, other high-value marine fish. Global Aquaculture Advocate Nov/Dec 2014:42–44

Bernard AM, Feldheim KA, Richards VP, Nemeth RS, Shivji MS (2012) Development and characterization of fifteen novel microsatellite loci for the Nassau grouper (*Epinephelus striatus*) and their utility for cross-amplification on a suite of closely related species. Conserv Genet Resour 4:983–986

Bolden SK (2000) Long-distance movement of a Nassau grouper (*Epinephelus striatus*) to a spawning aggregation in the central Bahamas. Fish Bull 98:642–645

Botsford LW, Brumbaugh DR, Grimes C, Kellner JB and others (2009) Connectivity, sustainability, and yield: bridging the gap between conventional fisheries management and marine protected areas. Rev Fish Biol Fish 19:69–95

Brumbaugh DR (2014) Guide to the science of marine protected areas in The Bahamas. American Museum of Natural History, New York, NY

Buchan KC (2000) The Bahamas. Mar Pollut Bull 41:94–111

Camp EF, Lohr KE, Barry SC, Bush PG, Jacoby SA, Manfrino C (2013) Microhabitat associations of late juvenile Nassau Grouper (*Epinephelus striatus*) off Little Cayman. Bull Mar Sci 89:571–581

Carter J, Marrow GJ, Pryor V (1991) Aspects of the ecology and reproduction of Nassau grouper, *Epinephelus striatus*, off the coast of Belize, Central America. Proc Gulf Caribb Fish Inst 43:65–111

Cheung WWL, Sarmiento JL, Dunne J, Frölicher TL and others (2012) Shrinking of fishes exacerbates impacts of global ocean changes on marine ecosystems. Nat Clim Change 3:254–258

Cheung WWL, Sadovy de Mitcheson Y, Braynen MT, Gittens LG (2013) Are the last remaining Nassau grouper *Epinephelus striatus* fisheries sustainable? Status quo in the Bahamas. Endang Species Res 20:27–39

Chiappone M, Sluka R, Sullivan Sealey K (2000) Groupers (Pisces: Serranidae) in fished and protected areas of the Florida Keys, Bahamas and northern Caribbean. Mar Ecol Prog Ser 198:261–272

Choat JH (2012) Spawning aggregations in reef fishes: ecological and evolutionary processes. In: Sadovy de Mitcheson Y, Colin PL (eds) Reef fish spawning aggregations: biology, research and management. Springer, New York, NY, p 85–116

Coleman FC, Koenig CC, Collins A (1996) Reproductive styles of shallow-water groupers (Pisces: Serranidae) in the eastern Gulf of Mexico and the consequences of fish spawning aggregations. Environ Biol Fishes 47:129–141

Coleman FC, Koenig CC, Huntsman GR, Musick JA and others (2000) Long-lived reef fishes: the grouper-snapper complex. Fisheries (Bethesda, Md) 25:14–21

Coleman FC, Koenig CC, Scanlon KM, Heppell S, Heppell S, Miller MW (2010) Benthic habitat modification through excavation by red grouper, *Epinephelus morio*, in the northeastern Gulf of Mexico. The Open Fish Sci J 3:1–15

Colin PL (1992) Reproduction of the Nassau grouper, *Epinephelus striatus* (Pisces: Serranidae) and its relationship to environmental conditions. Environ Biol Fishes 34: 357–377

Colin PL (1995) Surface currents in Exuma Sound, Bahamas and adjacent areas with reference to potential larval transport. Bull Mar Sci 56:48–57

Colin PL (1996) Longevity of some coral reef fish spawning aggregations. Copeia 1996:189–192

Colin PL (2012) Timing and location of aggregation and spawning in reef fishes. In: Sadovy de Mitcheson Y, Colin PL (eds) Reef fish spawning aggregations: biology, research and management. Springer, New York, NY, p 117–158

Colin PL, Laroche WA, Brothers EB (1997) Ingress and settlement in the Nassau grouper, *Epinephelus striatus* (Pisces: Serranidae), with relationship to spawning occurrence. Bull Mar Sci 60:656–667

Cornish A, Eklund AM (2003) *Epinephelus striatus*. The IUCN red list of threatened species, Version 2014.2. www.iucnredlist.org (accessed on 23 October 2014)

Cowen RK, Paris CB, Srinivasan A (2006) Scaling of connectivity in marine populations. Science 311:522–527

Craig MT, Hastings PA (2007) A molecular phylogeny of the groupers of the subfamily Epinephelinae (Serranidae) with a revised classification of the Epinephelini. Ichthyol Res 54:1–17

Craig MT, Heemstra PC, Sadovy YJ (2011) Groupers of the world: a field and market guide. National Inquiry Services Centre (NISC), Grahamstown

Cushion N, Sullivan-Sealey K (2008) Landings, effort and socio-economics of a small scale commercial fishery in The Bahamas. Proc Gulf Caribb Fish Inst 600:162–166

Cushion N, Cook M, Schull J, Sullivan-Sealey K (2008) Reproductive classification and spawning seasonality of *Epinephelus striatus* (Nassau grouper), *E. guttatus* (red hind) and *Mycteroperca venenosa* (yellowfin grouper) from The Bahamas. Proc 11th Int Coral Reef Symp, Ft Lauderdale, Florida 22:994–998

Dahlgren C (2004) Bahamian marine reserve: past experience and future plans. In: Sobel J, Dahlgren C (eds) Marine reserves: a guide to science, design and use. Island Press, Washington, DC, p 268–286

Dahlgren CP, Eggleston DB (2000) Ecological processes underlying ontogenetic habitat shifts in a coral reef fish. Ecology 81:2227–2240

Dahlgren CP, Eggleston DB (2001) Spatio-temporal variability in abundance, size and microhabitat associations of early juvenile Nassau grouper *Epinephelus striatus* in an off-reef nursery system. Mar Ecol Prog Ser 217:145–156

Dahlgren CP, Kellison GT, Adams AJ, Gillanders BM and others (2006) Marine nurseries and effective juvenile habitats: concepts and applications. Mar Ecol Prog Ser 312:291–295

Dahlgren C, Sherman K, Lang J, Kramer PR, Marks K (2016) Bahamas coral reef report card, Vol. 1: 2011–2013. Nassau, Bahamas, www.nature.org/media/bahamas/bahamas-coral-reef-report-card.pdf

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nat Rev Genet 12:499–510

Domeier ML (2012) Revisiting spawning aggregations: definitions and challenges. In: Sadovy de Mitcheson Y, Colin PL (eds) Reef fish spawning aggregations: biology, research and management. Springer, New York, NY, p 1–20

Eggleston DB (1995) Recruitment in Nassau grouper *Epinephelus striatus*: post-settlement abundance, microhabitat features, and ontogenetic habitat shifts. Mar Ecol Prog Ser 124:9–22

Eggleston DB, Grover JJ, Lipcius RN (1998) Ontogenetic diet shifts in Nassau grouper: trophic linkages and predatory impact. Bull Mar Sci 63:111–126

Ehrhardt NM, Deleveaux VKW (2007) The Bahamas' Nassau grouper (*Epinephelus striatus*) fishery—two assessment methods applied to a data-deficient coastal population. Fish Res 87:17–27

Eisbrenner WD, Botwright N, Cook M, Davidson EA and others (2014) Evidence for multiple sex-determining loci in Tasmanian Atlantic salmon (*Salmo salar*). Heredity 113:86–92

Ellis SC, Watanabe WO, Ellis EP (1997) Temperature effects on feed utilization and growth of postsettlement stage Nassau grouper. Trans Am Fish Soc 126:309–315

Espigares F, Rocha A, Molés G, Gómez A, Carrillo M, Zanuy S (2015) New insights into the factors mediating the onset of puberty in sea bass. Gen Comp Endocrinol 224: 176–185

Falahatkar B, Tolouei G, Mohammad H, Falahatkar S, Abbasalizadeh A (2011) Laparoscopy, a minimally-invasive technique for sex identification in cultured great sturgeon *Huso huso*. Aquaculture 321:273–279

FAO (Food and Agriculture Organization) (2014) The state of world fisheries and aquaculture opportunities and challenges 2014. FAO, Rome

Froese R, Pauly D (eds) (2014) FishBase. www.fishbase.org (accessed on 23 October 2014)

Gardner TA, Côté IM, Gill JA, Grant A, Watkinson AR (2003) Long-term region-wide declines in Caribbean corals. Science 301:958–960

Gascoigne J (2002) Nassau grouper and queen conch in The Bahamas: status and management options. MacAlister Elliot and Partners, Lymington, p 1–44, https://breef.org/wp-content/uploads/2015/03/BREEF-NGrouperQConchStatusReport2002.pdf

Griffiths AM, Machado-Schiaffino G, Dillane E, Coughlan J and others (2010) Genetic stock identification of Atlantic salmon (*Salmo salar*) populations in the southern part of the European range. BMC Genet 11:31

Grover JJ (1993) Trophic ecology of pelagic early-juvenile Nassau grouper, *Epinephelus striatus*, during an early phase of recruitment into demersal habitats. Bull Mar Sci 53:1117–1125

Grover JJ, Eggleston DB, Shenker JM (1998) Transition from pelagic to demersal phase in early-juvenile Nassau grouper, *Epinephelus striatus*: pigmentation, squamation and ontogeny of diet. Bull Mar Sci 62:97–113

Grüss A, Robinson J, Heppell SS, Heppell SA, Semmens BX (2014) Conservation and fisheries effects of spawning aggregation marine protected areas: What we know, where we should go, and what we need to get there. ICES J Mar Sci 71:1515–1534

Harikrishnan R, Balasundaram C, Heo MS (2011) Fish health aspects in grouper aquaculture. Aquaculture 320: 1–21

Harrison HB, Williamson DH, Evans RD, Almany GR and others (2012) Larval export from marine reserves and the recruitment benefit for fish and fisheries. Curr Biol 22: 1023–1028

Hateley JG (1995) Preliminary results of a protein electrophoretic analysis of genetic variation, population structure and gene flow in the Nassau grouper, *Epinephelus striatus*. Proc Gulf Caribb Fish Inst 47:888–905

Hauser L, Carvalho GR (2008) Paradigm shifts in marine fisheries genetics: ugly hypotheses slain by beautiful facts. Fish Fish 9:333–362

Heemstra PC, Randall JE (1993) FAO species catalogue, Vol. 16. Groupers of the world (family Serranidae, subfamily Epinephelinae): an annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper, and lyretail species known to date. FAO Fisheries Synopsis No. 125, Food and Agriculture Organization of the United Nations, Rome

Hemmer-Hansen J, Overgaard TN, Pujolar JM (2014) Population genomics of marine fishes: next-generation prospects and challenges. Biol Bull 227:117–132

Heppell SA, Sullivan CV (1999) Gag (*Mycteroperca microlepis*) vitellogenin: purification, characterization and use for enzyme-linked immunosorbent assay (ELISA) of female maturity in three species of grouper. Fish Physiol Biochem 20:361–374

Heppell SA, Semmens BX, Pattengill-Semmens CV, Bush PG and others (2009) Tracking potential larval dispersal patterns from Nassau grouper aggregation sites: evidence for local retention and the 'importance of place'. Proc Gulf Caribb Fish Inst 61:325–327

Heppell SA, Semmens BX, Pattengill-Semmens CV, Bush PG and others (2011) Oceanographic patterns associated with Nassau grouper aggregation spawn timing: shifts in surface currents on the nights of peak spawning. Proc Gulf Caribb Fish Inst 63:152–154

Heppell SA, Semmens BX, Archer SK, Pattengill-Semmens CV and others (2012) Documenting recovery of a spawning aggregation through size frequency analysis from underwater laser callipers measurements. Biol Conserv 155:119–127

Heyman WD, Kjerfve B (2008) Characterization of transient multi-species reef fish spawning aggregations at Gladden Spit, Belize. Bull Mar Sci 83:531–551

Heyman W, Requeña N (2002) Status of multi-species spawning aggregations in Belize. The Nature Conservancy, Punta Gorda

Hilborn R, Orensanz JM, Parma AM (2005) Institutions, incentives and the future of fisheries. Phil Trans R Soc B Biol Sci 360:47–57

Hohenlohe PA, Bassham S, Etter PD, Stiffler N, Johnson EA, Cresko WA (2010) Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. PLoS Genet 6:e1000862

Horreo JL, Machado-Schiaffino G, Ayllon F, Griffiths AM, Bright D, Stevens JR, Garcia-Vazquez E (2011) Impact of climate change and human-mediated introgression on southern European Atlantic salmon populations. Glob Change Biol 17:1778–1787

Huntsman GR, Potts J, Mays RW, Vaughan D (1999) Groupers (Serranidae, Epinephelinae): endangered apex predators of reef communities. In: Musick JA (ed) Life in the slow lane: ecology and conservation of long-lived marine animals. Am Fish Soc Symp 23:217–231

Hutchinson WF, Carvalho GR, Roger SI (2001) Marked genetic structuring in localised spawning populations of cod *Gadus morhua* in the North Sea and adjoining waters, as revealed by microsatellites. Mar Ecol Prog Ser 223:251–260

IUCN (International Union for Conservation of Nature) (2015) IUCN red list of threatened species, Version 2015-3. www.iucnredlist.org (accessed on 03 July 2015)

Jackson AM, Semmens BX, Bernardi G (2012) Characterization and cross-species amplification of microsatellite markers in Nassau grouper (*Epinephelus striatus*). Mol Ecol Resour 12:972–974

Jackson AM, Semmens BX, Sadovy de Mitcheson Y, Nemeth RS and others (2014) Population structure and phylogeography in Nassau grouper (*Epinephelus striatus*), a mass-aggregating marine fish. PLoS One 9:e97508

Jackson AM, Munguía-Vega A, Beldade R, Erisman BE, Bernardi G (2015) Incorporating historical and ecological genetic data for leopard grouper (*Mycteroperca rosacea*) into marine reserve design in the Gulf of California. Conserv Genet 16:811–822

Kadison E, Nemeth RS, Blondeau J, Smith T, Calnan J (2010) Nassau Grouper (*Epinephelus striatus*) in St. Thomas, US Virgin Islands, with evidence for a spawning aggregation site recovery. Proc Gulf Caribb Fish Inst 62:273–279

Kobara S, Heyman WD (2008) Geomorphometric patterns of Nassau grouper (*Epinephelus striatus*) spawning aggregation sites in the Cayman Islands. Mar Geod 31: 231–245

Kobara S, Heyman WD, Pittman SJ, Nemeth RS (2013) Biogeography of transient fish spawning aggregations in the Caribbean: a synthesis for future research and management. Oceanogr Mar Biol Annu Rev 51:281–326

Kough AS, Paris CB, Butler MJIV (2013) Larval connectivity and the international management of fisheries. PLoS One 8:e64970

Lindeman KC, Pugliese R, Waugh GT, Ault JS (2000) Developmental patterns within a multispecies reef fishery: management applications for essential fish habitats and protected areas. Bull Mar Sci 66:929–956

Lotze HK, Lenihan HS, Bourque BJ, Bradbury RH and others (2006) Depletion, degradation, and recovery potential of estuaries and coastal seas. Science 312:1806–1809

Luckhurst BE (2001) Policies, legislation and management options: workshop report 'working towards sustainable management of Nassau groupers in Belize'. Belize City, 30 July, 2001. Green Reef Environmental Institute, Belize City

Luo YS, Hu W, Liu XC, Lin HR, Zhu ZY (2010) Molecular cloning and mRNA expression pattern of *Sox9* during sex reversal in orange-spotted grouper (*Epinephelus coioides*). Aquaculture 306:322–328

Maggio T, Andaloro F, Arculeo M (2006) Genetic population structure of *Epinephelus marginatus* (Pisces, Serranidae) revealed by two molecular markers. Ital J Zool 73: 275–283

Matsche MA, Bakal RS, Rosemary KM (2011) Use of laparoscopy to determine sex and reproductive status of shortnose sturgeon (*Acipenser brevirostrum*) and Atlantic sturgeon (*Acipenser oxyrinchus oxyrinchus*). J Appl Ichthyology 27:627–636

Matsuda M, Matsuda C, Hamaguchi S, Sakaizumi M (1998) Identification of the sex chromosomes of the medaka, *Oryzias latipes*, by fluorescence *in situ* hybridization. Cytogenet Cell Genet 82:257–262

Molloy PP, Côté IM, Reynolds JD (2012) Why spawn in aggregations? In: Sadovy de Mitcheson Y, Colin PL (eds) Reef fish spawning aggregations: biology, research and management, Springer, New York, NY, p 57–84

Moritz C (2002) Strategies to protect biological diversity and the evolutionary processes that sustain it. Syst Biol 51: 238–254

Morris A, Roberts C, Hawkins J (2000) The threatened status of groupers (Epinephelinae). Biodivers Conserv 9: 919–942

Moultrie S (2012) Master plan for The Bahamas national protected area system. The Nature Conservancy, Northern Caribbean Office, Nassau

Mumby PJ, Dahlgren CP, Harborne AR, Kappel CV and others (2006) Fishing, trophic cascades, and the process of grazing on coral reefs. Science 311:98–101

Mumby PJ, Steneck RS, Edwards AJ, Ferrari R, Coleman R, Harborne AR, Gibson JP (2012) Fishing down a Caribbean food web relaxes trophic cascades. Mar Ecol Prog Ser 445:13–24

Muñoz RC, Currin CA, Whitfield PE (2011) Diet of invasive lionfish on hard bottom reefs of the Southeast USA: insights from stomach contents and stable isotopes. Mar Ecol Prog Ser 432:181–193

Murua H, Saborido-Rey F (2003) Female reproductive strategies of marine fish species of the North Atlantic. J Northwest Atl Fish Sci 33:23–31

Musick JA (1999) Criteria to define extinction risk in marine fishes: the American Fisheries Society Initiative. Fisheries (Bethesda, Md) 24:6–14

Narum SR, Buerkle CA, Davey JW, Miller MR, Hohenlohe PA (2013) Genotyping-by-sequencing in ecological and conservation genomics. Mol Ecol 22:2841–2847

Nielsen EE, Hemmer-Hansen J, Larsen PF, Bekkevold D (2009) Population genomics of marine fishes: identifying adaptive variation in space and time. Mol Ecol 18: 3128–3150

Palumbi SR (2004) Marine reserves and ocean neighborhoods: the spatial scale of marine populations and their management. Annu Rev Environ Resour 29:31–68

Paris CB, Helgers J, van Sebille E, Srinivasan A (2013) Connectivity modelling system: a probabilistic modelling tool for the multi-scale tracking of biotic and abiotic variability in the ocean. Environ Model Softw 42:47–54

Paris JR, King RA, Stevens JR (2015) Human mining activity across the ages determines the genetic structure of modern brown trout (*Salmo trutta* L.) populations. Evol Appl 8:573–585

Patterson HM, Thorrold SR, Shenker JM (1999) Analysis of otolith chemistry in Nassau grouper (*Epinephelus striatus*) from The Bahamas and Belize using solution-based ICP-MS. Coral Reefs 18:171–178

Pittman SJ, Monaco ME, Friedlander AM, Legare B and others (2014) Fish with chips: tracking reef fish movements to evaluate size and connectivity of Caribbean marine protected areas. PLoS One 9:e96028

Ray C (1998) Bahamian protected areas part 1: how it all began. Bahamas J Sci 6:2–10

Ray GC (2000) Investigations of Nassau grouper breeding aggregations at High Cay, Andros: implications for a conservation strategy. Final Report, Department of Fisheries, Nassau

Reiss H, Hoarau G, Dickey-Collas M, Wolff WJ (2009) Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish Fish 10:361–395

Rivera MAJ, Kelley CD, Roderick GK (2004) Subtle population genetic structure in the Hawaiian grouper, *Epinephelus quernus* (Serranidae) as revealed by mitochondrial DNA analyses. Biol J Linn Soc 81:449–468

Rivera MAJ, Andrews KR, Kobayashi DR, Wren JLK, Kelley C, Roderick GK, Toonen RJ (2011) Genetic analyses and simulations of larval dispersal reveal distinct populations and directional connectivity across the range of the Hawaiian Grouper (*Epinephelus quernus*). J Mar Biol 2011:1–11

Roberts CM, Quinn N, Tucker JW, Woodward PN (1995) Introduction of hatchery-reared Nassau grouper to a coral reef environment. N Am J Fish Manage 15:159–164

Robinson J, Samoilys MA (2013) Reef fish spawning aggregations in the Western Indian Ocean: research for management. WIOMSA Book Series 13. WIOMSA, CORDIO, Nairobi

Robinson J, Cinner JE, Graham NA (2014) The influence of fisher knowledge on the susceptibility of reef fish aggregations to fishing. PLoS One 9:e91296

Rowell TJ, Nemeth RS, Schärer MT, Appeldoorn RS (2015) Fish sound production and acoustic telemetry reveal behaviors and spatial patterns associated with spawning aggregations of two Caribbean groupers. Mar Ecol Prog

Ser 518:239–254
Rudd MA, Tupper MH (2002) The impact of Nassau grouper size and abundance on scuba diver site selection and MPA economics. Coast Manage 30:133–151
Russell MW, Sadovy de Mitcheson Y, Erisman BE, Hamilton RJ, Luckhurst BE, Nemeth RS (2014) Status report: world's fish aggregations 2014. Science and Conservation of Fish Aggregations, California, USA. International Coral Reef Initiative
Sadovy Y, Colin PL (1995) Sexual development and sexuality in the Nassau grouper. J Fish Biol 46:961–976
Sadovy Y, Domeier M (2005) Are aggregation-fisheries sustainable? Reef fish fisheries as a case study. Coral Reefs 24:254–262
Sadovy Y, Eklund AM (1999) Synopsis of biological data on the Nassau grouper, *Epinephelus striatus* (Bloch, 1792) and the Jewfish, *E. itajara* (Lichtenstein, 1822). NOAA Tech Rep NMFS 146
Sadovy de Mitcheson Y, Colin PL (eds) (2012) Reef fish spawning aggregations: biology, research and management, Vol. 35: fish and fisheries. Springer, New York, NY
Sadovy de Mitcheson YS, Erisman B (2012) Fishery and biological implications of fishing spawning aggregations, and the social and economic importance of aggregating fishes. In: Sadovy de Mitcheson Y, Colin PL (eds) Reef fish spawning aggregations: biology, research and management. Springer, New York, NY, p 225–284
Sadovy de Mitcheson Y, Cornish A, Domeier M, Colin PL, Russell M, Lindeman KC (2008) A global baseline for spawning aggregations of reef fishes. Conserv Biol 22: 1233–1244
Sadovy de Mitcheson Y, Craig MT, Bertoncini AA, Carpenter KE and others (2013) Fishing groupers towards extinction: a global assessment of threats and extinction risks in a billion dollar fishery. Fish Fish 14:119–136
Sala E, Ballesteros E, Starr RM (2001) Rapid decline of Nassau grouper spawning aggregations in Belize: fishery management and conservation needs. Fisheries (Bethesda, Md) 26:23–30
Schärer MT, Nemeth MI, Appeldoorn RS (2010) Protecting a multi-species spawning aggregation at Mona Island, Puerto Rico. Proc Gulf Caribb Fish Inst 62:252–259
Schärer MT, Rowell TJ, Nemeth MI, Appledoorn RS (2012) Sound production associated with reproductive behavior of Nassau grouper *Epinephelus striatus* at spawning aggregations. Endang Species Res 19:29–38
Schärer-Umpierre M, Nemeth R, Tuohy E, Clouse K, Nemeth M, Appledoorn RS (2014) Nassau grouper *Epinephelus striatus* fish spawning aggregations in the US Caribbean. Proc Gulf Caribb Fish Inst 66:408–412
Schoelinck C, Hinsinger D, Dettaï A, Cruaud C, Justine JL (2014) A phylogenetic re-analysis of groupers with applications for ciguatera fish poisoning. PLoS One 9:e98198
Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol Lett 9:615–629
Semmens BX, Luke KE, Bush PG, Pattengill-Semmens C, Johnson B, McCoy C, Heppell S (2007) Investigating the reproductive migration and spatial ecology of Nassau grouper (*Epinephelus striatus*) on Little Cayman Island using acoustic tags — an overview. Proc Gulf Caribb Fish Inst 58:199–206
Semmens BX, Bush P, Heppell S, Johnson B, McCoy C, Pattengill-Semmens C, Whaylen L (2008) Charting a course for Nassau grouper recovery in the Caribbean: what we've learned and what we still need to know. Proc Gulf Caribb Fish Inst 60:607–609
Shapiro DY (1987) Reproduction in groupers. In: Polvina JJ, Ralston SR (eds) Tropical snappers and groupers: biology and fisheries management. Westview Press, Boulder, CO, p 295–327
Shenker JM, Maddox ED, Wishinski E, Pearl A, Thorrold SR, Smith N (1993) Onshore transport of settlement-stage Nassau grouper *Epinephelus striatus* and other fishes in Exuma Sound, Bahamas. Mar Ecol Prog Ser 98:31–43
Sherman KD, Dahlgren CP, Brumbaugh DR, Knowles LC (2013) Tourism and coral reef health in the Exuma Cays Land and Sea Park. Proc Gulf Caribb Fish Inst 65: 234–242
Shikano T, Herczeg G, Merilä J (2011) Molecular sexing and population genetic inference using a sex-linked microsatellite marker in the nine-spined stickleback (*Pungitius pungitius*). BMC Res Notes 4:119
Shulman MJ, Bermingham E (1995) Early life histories, ocean currents, and the population genetics of Caribbean reef fishes. Evolution 49:897–910
Silva-Oliveira GC, do Rêgo PS, Schneider H, Sampaio I, Vallinoto M (2008) Genetic characterisation of populations of the critically endangered Goliath grouper (*Epinephelus itajara*, Serranidae) from the Northern Brazilian coast through analyses of mtDNA. Genet Mol Biol 31: 988–994
Sluka R, Chiappone M, Sullivan KM, Garine-Wichatitsky D (1993) Benthic habitat characterization and space utilization by juvenile epinepheline groupers in the Exuma Cays Land and Sea Park, Central Bahamas. Proc Gulf Caribb Fish Inst 45:23–36
Sluka R, Chiappone M, Sullivan KM, Wright R (1996) Habitat and life in the Exuma Cays, The Bahamas: the status of groupers and coral reefs in the Northern Cays. Media Publishing, Nassau
Sluka R, Chiappone M, Sullivan KM, Wright R (1997) The benefits of a marine fishery reserve for Nassau Grouper *Epinephelus striatus* in the Central Bahamas. Proc 8th Int Coral Reef Symp 2:1961–1964
Smith CL (1972) A spawning aggregation of Nassau grouper, *Epinephelus striatus* (Bloch). Trans Am Fish Soc 101:257–261
Smith PJ, Francis RICC, McVeagh M (1991) Loss of genetic diversity due to fishing pressure. Fish Res 10:309–316
Stallings CD (2008) Effects of an exploited predator on recruitment of coral-reef fishes. Ecology 89:2090–2095
Stallings CD (2009) Fishery-independent data reveal negative effect of human population density on Caribbean predatory fish communities. PLoS One 4:e5333
Starr RM, Sala E, Ballesteros E, Zabala M (2007) Spatial dynamics of the Nassau grouper *Epinephelus striatus* in a Caribbean atoll. Mar Ecol Prog Ser 343:239–249
Stevens-McGeever SJ, Semmens BX, Pattengill-Semmens C, Heppell S, Bush P, Johnson B, McCoy C (2013) Alterations in home range space over a multi-year study of Nassau grouper (*Epinephelus striatus*). Proc Gulf Caribb Fish Inst 65:204
Stevenson DE, Champman RW, Sedberry GR (1998) Stock identification in Nassau grouper, *Epinephelus striatus*, using microsatellite DNA analysis. Proc Gulf Caribb Fish Inst 50:727–749
Sunday JM, Calosi P, Dupont S, Munday PL, Stillman JH, Reusch TBH (2014) Evolution in an acidifying ocean. Trends Ecol Evol 29:117–125

Sunnucks P (2000) Efficient genetic markers for population biology. Trends Ecol Evol 15:199–203

Takemura A, Rahman MS, Park YJ (2010) External and internal controls of lunar-related reproductive rhythms in fishes. J Fish Biol 76:7–26

Teng Y, Hou Z, Gong J, Liu H and others (2008) Whole-genome transcriptional profiles of a novel marine fish iridovirus, Singapore grouper iridovirus (SGIV) in virus-infected grouper spleen cell cultures and in orange-spotted grouper, *Epinephulus coioides*. Virology 377: 39–48

Tucker JW Jr (1999) Grouper aquaculture. Southern Regional Aquaculture Center Publication No. 721, Stoneville, MS

Tucker JW Jr, Woodard PC (1991) Growth and development of domestic juvenile Nassau groupers. Proc Gulf Caribb Fish Inst 43:389–391

Tyler CR, Jobling S (2008) Roach, sex, and gender-bending chemicals: the feminization of wild fish in English rivers. Bioscience 58:1051–1059

Vo AE, Ashley MC, Dikou A, Newman SP (2014) Fishery exploitation and stock assessment of the endangered Nassau Grouper, *Epinephelus striatus* (Actinopterygii: Perciformes: Serranidae) in the Turks and Caicos Islands. Acta Ichthyol Piscat 44:117–122

Wang L, Liu S, Zhuang Z, Guo L, Meng Z, Lin H (2013) Population genetic studies revealed local adaptation in a high gene-flow marine fish, the small yellow croaker (*Larimichthys polyactis*). PLoS One 8:e83493

Watanabe WO, Ellis SC, Ellis EP, Head WD and others (1995a) Progress in controlled breeding of Nassau grouper (*Epinephelus striatus*) broodstock by hormone induction. Aquaculture 138:205–219

Watanabe WO, Lee CS, Ellis SC, Ellis EP (1995b) Hatchery study of the effects of temperature on eggs and yolksac larvae of the Nassau grouper *Epinephelus striatus*. Aquaculture 136:141–147

Watson AC, Siemann LA, Hanlon RT (2014) Dynamic camouflage by Nassau groupers *Epinephelus striatus* on a Caribbean coral reef. J Fish Biol 85:1634–1649

Western Central Atlantic Fishery Commission (2014) The first meeting of the CFMC/WECAFC/OSPESCA/CRFM working group on spawning aggregations, Report No. 1059. FAO Fisheries and Aquaculture, Bridgetown

Whaylen L, Pattengill-Semmens CV, Semmens BX, Bush PG, Boardmand MR (2004) Observations of a Nassau grouper, *Epinephelus striatus*, spawning aggregation site in Little Cayman, Cayman Islands, including multi-species spawning information. Environ Biol Fishes 70: 305–313

Whaylen L, Bush PG, Johnson BC, Luke KE and others (2007) Aggregation dynamics and lessons learned from five years of monitoring at a Nassau grouper (*Epinephelus striatus*) spawning aggregation in Little Cayman, Cayman Islands, BWI. Proc Gulf Caribb Fish Inst 59: 413–422

White JW (2015) Marine reserve design theory for species with ontogenetic migration. Biol Lett 11:20140511

Whiteman EA, Jennings CA, Nemeth RS (2005) Sex structure and potential female fecundity in a *Epinephelus guttatus* spawning aggregation: applying ultrasonic imaging. J Fish Biol 66:983–995

Wilson RS, Hardisty DJ, Epanchin-Neill RS, Runge MC and others (2016) A typology of time-scale mismatches and behavioral interventions to diagnose and solve conservation problems. Conserv Biol 30:42–49

Young JL, Bornik ZB, Marcotte ML, Charlie KN, Wagner GN, Hinch SG, Cooke SJ (2006) Integrating physiology and life history to improve fisheries management and conservation. Fish Fish 7:262–283

Zatcoff MS, Ball AO, Sedberry GR (2004) Population genetic analysis of red grouper, *Epinephelus morio*, and scamp, *Mycteroperca phenax*, from the southeastern U.S. Atlantic and Gulf of Mexico. Mar Biol 144:769–777

Editorial responsibility: Inna Sokolova, Charlotte, North Carolina, USA

Submitted: December 22, 2015; Accepted: May 11, 2016
Proofs received from author(s): July 5, 2016

Chapter II

Historical processes and contemporary anthropogenic activities influence genetic population dynamics of Nassau grouper (*Epinephelus striatus*) within The Bahamas

Manuscript published in Frontiers in Marine Science

Authors: Sherman KD, King RA, Dahlgren CP, Simpson SD, Stevens JR, Tyler CR

frontiers in Marine Science

ORIGINAL RESEARCH
published: 06 December 2017
doi: 10.3389/fmars.2017.00393

Historical Processes and Contemporary Anthropogenic Activities Influence Genetic Population Dynamics of Nassau Grouper (*Epinephelus striatus*) within The Bahamas

Krista D. Sherman[1,2,3], R. Andrew King[1], Craig P. Dahlgren[3,4], Stephen D. Simpson[1], Jamie R. Stevens[1] and Charles R. Tyler[1]**

[1] Biosciences, University of Exeter, Exeter, United Kingdom, [2] Daniel P. Haerther Center for Conservation and Research, John G. Shedd Aquarium, Chicago, IL, United States, [3] Science and Policy, Bahamas National Trust, Nassau, Bahamas, [4] Perry Institute for Marine Science, Waitsfield, VT, United States

OPEN ACCESS

Edited by:
Sophie von der Heyden,
Stellenbosch University, South Africa

Reviewed by:
Giacomo Bernardi,
University of California, Santa Cruz, United States
Romina Henriques,
Stellenbosch University, South Africa

***Correspondence:**
Jamie R. Stevens
j.r.stevens@exeter.ac.uk
Charles R. Tyler
c.r.tyler@exeter.ac.uk

Specialty section:
This article was submitted to Marine Conservation and Sustainability, a section of the journal Frontiers in Marine Science

Received: 08 September 2017
Accepted: 22 November 2017
Published: 06 December 2017

Citation:
Sherman KD, King RA, Dahlgren CP, Simpson SD, Stevens JR and Tyler CR (2017) Historical Processes and Contemporary Anthropogenic Activities Influence Genetic Population Dynamics of Nassau Grouper (Epinephelus striatus) within The Bahamas. Front. Mar. Sci. 4:393. doi: 10.3389/fmars.2017.00393

Severe declines of endangered Nassau grouper (*Epinephelus striatus*) across The Bahamas and Caribbean have spurred efforts to improve their fisheries management and population conservation. The Bahamas is reported to hold the majority of fish spawning aggregations for Nassau grouper, however, the status and genetic population structure of fish within the country is largely unknown, presenting a major knowledge gap for their sustainable management. Between August 2014–February 2017, 464 individual Nassau grouper sampled from The Bahamas were genotyped using 15 polymorphic microsatellite loci to establish measures of population structure, genetic diversity and effective population size (N_e). Nassau grouper were characterized by mostly high levels of genetic diversity, but we found no evidence for geographic population structure. Microsatellite analyses revealed weak, but significant genetic differentiation of Nassau grouper throughout the Bahamian archipelago (Global F_{ST} 0.00236, $p = 0.0001$). Temporal analyses of changes in N_e over the last 1,000 generations provide evidence in support of a pronounced historic decline in Bahamian Nassau grouper that appears to pre-date anthropogenic fishing activities. M-ratio results corroborate significant reductions in N_e throughout The Bahamas, with evidence for population bottlenecks in three islands and an active fish spawning aggregation along with apparent signs of inbreeding at two islands. Current estimates of N_e for Nassau grouper are considerably lower compared with historic levels. These findings represent important new contributions to our understanding of the evolutionary history, demographics and genetic connectivity of this endangered species, which are of critical importance for advancing their sustainable management.

Keywords: bottleneck, connectivity, effective population size, endangered species, fish spawning aggregation, genetic diversity, microsatellites

INTRODUCTION

Anthropogenic activities, most notably the over-exploitation of species and habitat degradation has led to significant global declines in biodiversity and genetic diversity (Worm et al., 2006; Allendorf et al., 2008; Paddack et al., 2009; Pinsky and Palumbi, 2014). Understanding population status, demographics and patterns of connectivity is vital from a management perspective to conserve ecologically and economically important species. Species that reproduce via fish spawning aggregations (FSAs; Domeier, 2012) are particularly susceptible to unsustainable fishing and many of these species are not only commercially valuable, but also provide important marine ecosystem services (Coleman et al., 2010; Sadovy de Mitcheson et al., 2013; Archer et al., 2015). Nassau grouper (*Epinephelus striatus*, Bloch, 1792) are among the top predatory reef fish species, naturally distributed throughout the Tropical Western Atlantic including Bermuda, Florida, The Bahamas and Yucatan Peninsula, the Caribbean Sea and parts of the Gulf of Mexico (Heemstra and Randall, 1993; Albins et al., 2009; Froese and Pauly, 2014). However, they have experienced severe population declines and even local extirpations throughout their native range over recent decades (Olsen and LaPlace, 1979; Sadovy and Eklund, 1999; Sadovy De Mitcheson et al., 2008).

The highly synchronous and predictable nature of FSAs is often associated with targeted fishing activity (Smith, 1972; Aguilar-Perera, 2006). FSA fishing has been identified as one of the leading drivers for significant reductions in Nassau grouper abundance (Sadovy De Mitcheson et al., 2008; Sadovy de Mitcheson et al., 2013; Cheung et al., 2013). While profitable for fishers (Cheung et al., 2013), this practice heavily reduces reproductive fish biomass and is detrimental to the integrity of Nassau grouper stocks (Sala et al., 2001; Sadovy and Domeier, 2005; Aguilar-Perera, 2006; Sadovy de Mitcheson et al., 2013). Global declines in Nassau grouper abundance and the disappearance of historic FSAs have led to IUCN-Red List and United States Endangered Species Act designations of endangered and threatened respectively (Cornish and Eklund, 2003; Albins et al., 2009). This has helped to focus conservation management efforts with fisheries management regulations now implemented in a number of countries where Nassau grouper still exist, with recent reports from the Cayman Islands and the United States Virgin Islands of population recovery (USVI; Kadison et al., 2010; Heppell et al., 2012). More generally, however, stock assessment data are deficient and marked declines in commercial landings, FSA abundance and densities on reef habitats have been documented in The Bahamas despite regulatory policies (Cheung et al., 2013; Dahlgren et al., 2016b; Sherman et al., 2016; Stump et al., 2017).

Advances in molecular biology and population genetics have proven to be extremely valuable in generating information on population status, genetic diversity and connectivity of a variety of fish species (Carvalho and Hauser, 1998; Silva-Oliveira et al., 2008; Davey et al., 2011; Adams et al., 2016; Garcia-Mayoral et al., 2016); in turn, these data have been applied to support population management and conservation efforts (Waples et al., 2008; Beldade et al., 2014; Selkoe et al., 2016). In particular, the application of genetics for stock identification (Carvalho and Hauser, 1994) and estimates of effective population size, N_e (Wright, 1931), combined with traditional fisheries stock assessment models (Hilborn and Walters, 1992) are emerging approaches for advancing conservation management of at-risk species (Luikart et al., 2010; Hare et al., 2011; Ovenden et al., 2016). Effective population size is a particularly informative measure for genetic diversity studies because it accounts for genetic drift and inbreeding. Reduced genetic diversity and N_e are often indicative of a population bottleneck, which may reduce adaptive potential and exacerbate extinction risk, especially in vulnerable species (Smith et al., 1991; Hauser et al., 2002; Luikart et al., 2010; Hare et al., 2011).

Previous assessments of the population genetic dynamics of Nassau grouper have primarily focused on the wider Caribbean (Hateley, 1995; Stevenson et al., 1998; Jackson A. M. et al., 2014; Bernard et al., 2016), with reduced representation (i.e., both in sample size and spatial coverage) from The Bahamas. Jackson A. M. et al. (2014) provided arguments for regional genetic subdivision based upon analyses using multiple molecular makers [i.e., mtDNA, microsatellites and single nucleotide polymorphisms (SNPs)] applied to samples collected throughout the Caribbean and The Bahamas. The authors also suggested that The Bahamas may be genetically distinct, with the Exumas representing a barrier to gene flow within The Bahamas, and between The Bahamas and the Caribbean. More recently, Bernard et al. (2016) explored genetic differentiation from two geographically disparate FSAs in the Caribbean and found no significant difference (F_{ST}=−0.0004) between FSAs in the Cayman Island and USVI, with low relatedness of Nassau grouper within the USVI. Based on these findings, the authors deduced that external rather than local recruitment was responsible for maintenance of the USVI FSA (Bernard et al., 2016).

The life history and ecological characteristics of Nassau grouper (Sadovy and Colin, 1995; Sadovy and Eklund, 1999) coupled with its economic value make it an ideal model species to demonstrate the value of applying population genetics to the conservation management of endangered and FSA targeted species. The Bahamas contains the majority (50–60%) of Nassau grouper FSAs globally, but the status of spawning stocks is poorly known and there is a paucity of information on the genetic composition of these fish (Sherman et al., 2016).

In the present study, we aimed to resolve whether Bahamian populations of Nassau grouper are indeed genetically distinct and to explore how anthropogenic activities may have influenced the genetic architecture of contemporary stocks. Specifically, our objectives were: to (1) assess the current status, genetic population structure and dynamics of Nassau grouper in The Bahamas; (2) estimate the effective population size of Bahamian Nassau grouper; (3) ascertain whether bottlenecks have occurred, which may compromise the genetic health of contemporary populations; and (4) based on these findings, to provide management recommendations for supporting their recovery.

METHODS

Study Area and Sample Collection

The Bahamas is an archipelagic nation consisting of 700 islands and 3,000 cays located in the tropical Western Atlantic (~80.5 km/50 mi) southeast of the United States of America and north of Cuba (Buchan, 2000; **Figure 1**). A total of 464 Nassau grouper fin clip samples were collected from 13 islands (n = 407) and one active FSA in The Bahamas (n = 57) between August 2014–February 2017 (**Figure 1**, **Table 1**). Tissue samples from living fish (n = 186) were collected with permission from the Department of Marine Resources during 10 research cruises (see Acknowledgements). Nassau grouper were captured via fish traps or divers using hand-held nets in water depths ranging from 4.6 to 32 m. Fish were slowly brought to the surface and processed using methods described by Stump et al. (2017). Nassau grouper were Floy™ tagged, measured (standard length, SL and total length, TL to the nearest 0.1 centimeter), and a small (~5 × 5 mm) tissue sample was removed from the anal or dorsal fin. Each fish was weighed (to the nearest 0.1 kg) and allowed to recover. Fish were then individually escorted back to the reef from which they were captured and monitored for 1–2 min to ensure there were no adverse impacts due to handling. Fin clips from recently killed specimens (n = 280) were also opportunistically collected from local fishing docks and landing sites throughout The Bahamas. If the capture location for a fish was not specified or was ambiguous, these individuals were treated as unknown. Samples were preserved in 95–100% ethanol (n = 452) or DMSO (n = 14) prior to genomic DNA extraction.

DNA Extraction, PCR and Genotyping

Genomic DNA for microsatellite analysis was extracted from fin clip tissues (n = 464) using the HotSHOT method (Truett et al., 2000) and its quality assessed via NanoDrop Spectrophotometry (ND−1,000). Twelve species-specific and three cross-species grouper microsatellite loci: Est33a, Est416, Est360, Est92, Est265, Est376, Est267, Est420, Est338, Est340, Est290, Est262, EACD08, EACB6, and EACD02 developed by Bernard et al. (2012) and Jackson et al. (2012) were selected for this research. Polymerase Chain Reaction (PCR) amplification was performed using a BIO-RAD MyCycler Thermal Cycler® in a total of three multiplexes

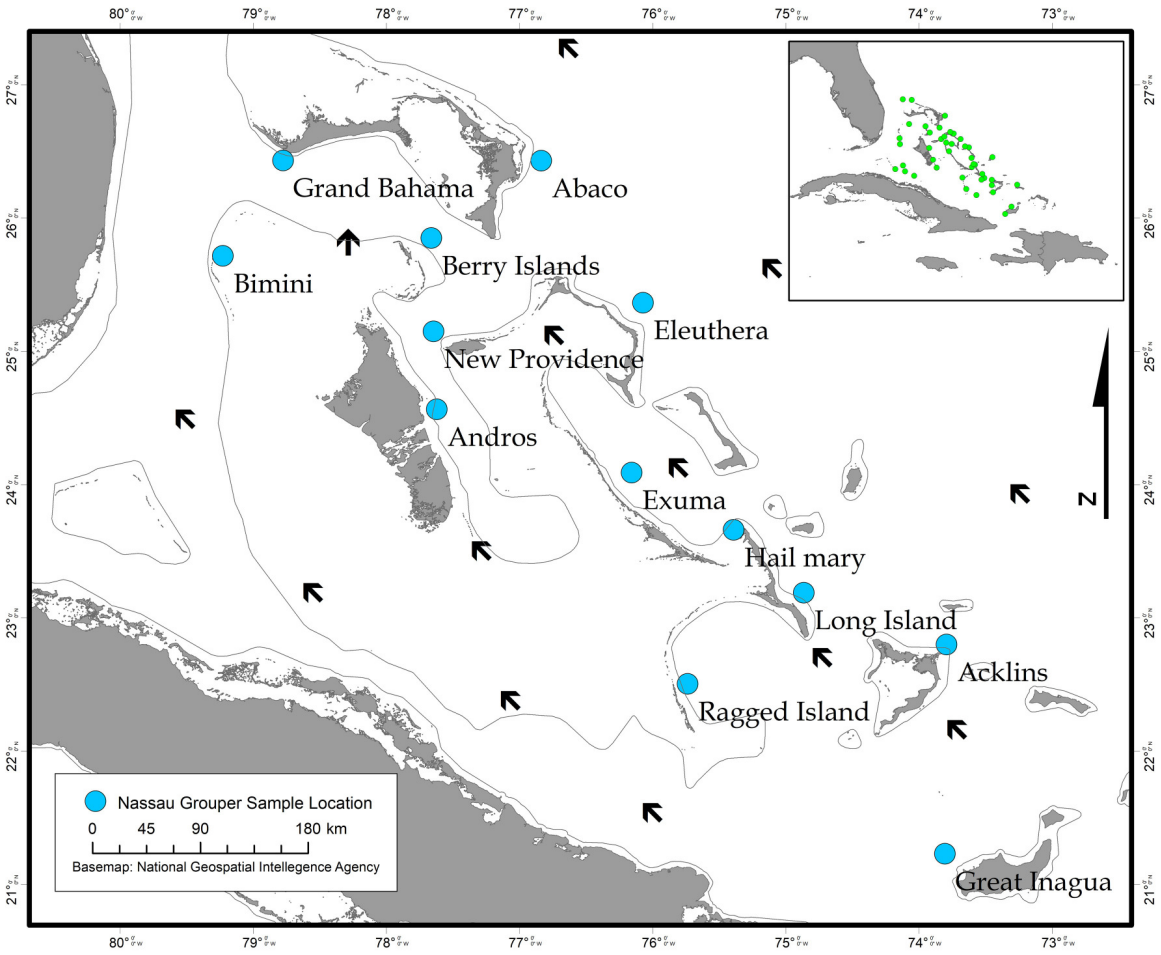


FIGURE 1 | Map showing sampling locations of Nassau grouper collected in The Bahamas (blue circles) and prevailing currents (black arrows), with an inset map depicting approximate locations of in-country Nassau grouper fish spawning aggregations (FSAs) (green circles).

TABLE 1 | Sampling information for Nassau grouper specimens including a breakdown of the number of fin clips = N, number of genotypes = N_g, life stages for genotyped fish, and the mean total length (TL) ± standard deviation (SD) of fish measured in each location.

Location	Location code	Collection period	N	N_g	Adults (≥48 cm TL)	Sub-adults (15–47 cm TL)	Juveniles (3–14 cm TL)	Unknown life stage	Mean TL (±SD)
Bimini	BIM	2015	1	1	1		–		49
Grand Bahama	GB	2015–2017	6	5			–	5	–
Abaco	AB	2016	78	75		65	–	10	31.1 (±6.2)
New Providence	NP	2016	11	10	2	8	–		28.7 (±13.8)
Berry Islands		2015	8	8	6	2	–		51.0 (±13.3)
Andros	AN	2014–2016	51	50	44	2	–	4	59.0 (±7.0)
Eleuthera	EL	2015–2016	64	63	30	29	–	4	49.7 (±14.8)
Exuma	EX	2016	44	44	19	25	–		43.8 (±11.0)
Hail Mary	HM	2014–2017	57	57	49	6	–	2	60.3 (±4.0)
Long Island	LI	2015–2016	22	21	12	8	–	1	46.5 (±1.9)
Cat Island	CI	2016	7	7	7		–		73.7 (±14.0)
Acklins	AK	2016	5	5			–	5	–
Ragged Island	RI	2016	9	7		7	–		27.6 (±14.5)
Great Inagua	GI	2016	46	46			–	46	–
Unknown	UK	2016	55	55	28	2	–	25	58.0 (±5.3)
Totals			464	454	198	154	–	102	

each containing 1 μl DNA, 3 μl of RNase-free water and 5 μl of Qiagen HotStarTaq plus MasterMix, and 1 μl of primer mixture under identical touchdown thermal cycling conditions (Supplementary Tables 1, 2; Hamilton and Tyler, 2008).

Amplified PCR products were visualized and scored with a Beckman Coulter CEQ^{TM} 8,000 sequencer (Fullerton, California, USA) using the manufacturer specified internal size standard. Genotypes with more than four missing loci (i.e., 10 individuals) were excluded from further analyses. Microsatellite loci were assessed for the presence of null alleles, large allele dropout, scoring error and stutter using Microchecker v.2.2.3 (van Oosterhout et al., 2004; Supplementary Table 3). The log-likelihood ratio statistic and probability test in GENEPOP v.4.2 was used to test for linkage disequilibrium and conformance to Hardy-Weinberg equilibrium (Rousset, 2008). To reveal Type I errors in both tests, the false discovery rate (FDR) correction was also applied (Storey and Tibshirani, 2003).

Genetic Diversity

To address whether patterns of genetic composition were due to temporal and/or Wahlund effects (i.e., heterozygosity deficiency due to unknown/cryptic population sub-structuring), we computed a range of genetic diversity parameters for Nassau grouper by sampling year (2014–2016) and for sub-adult and adult fish from two locations (Eleuthera and Exuma) with relatively robust sample sizes (Supplementary Table 4). Specifically, observed (H_O) and expected heterozygosity (H_E) were calculated using GenAlEx v.6.503 (Peakall and Smouse, 2006, 2012). The rarefaction method in HP-Rare v. 1.1 (Kalinowski, 2005) was used to calculate allelic richness (A_R) and private allelic richness (PA_R) for 2014, 2015, 2016, sub-adult and adult fish with minimum sample sizes of 14, six, 10, 14, and 14 genes per locus respectively. Because these measures produced similar results (Supplementary Table 4), data was pooled for subsequent analyses. Genetic diversity statistics including gene diversity and allelic richness were determined using microsatellite profiles for 454 fish (**Table 2**). A_R and PA_R for the pooled data set was computed with a minimum sample size of 14 genes for each location. Significant differences in genetic diversity parameters (H_O, H_E, A_R, and PA_R) among sample locations were calculated using the non-parametric Kruskal-Wallis rank sum test in R v.3.4.0.

Ascertainment bias was not a consideration in the comparison of our diversity results with those of Jackson A. M. et al., 2014. The current study had three loci in common with that of Jackson A. M. et al., 2014: EACB6, EACD02, and EACD08; these loci were originally designed for another grouper species (Gulf coney-*Hyporthodus acanthistius*) and successfully cross-amplified for Nassau grouper (Jackson et al., 2012). In both our study and that of Jackson A. M. et al., 2014 these loci were used with the same species (Nassau grouper).

Genetic Differentiation and Demographic Analyses

Locations with sample sizes of ≥10 individuals were used in assessments of genetic differentiation, corresponding to a total of 421 Nassau grouper sampled from Abaco in the north to Great Inagua in the south. Significant differences in genetic differentiation (i.e., global and pairwise F_{ST} values; Weir and Cockerham, 1984) were computed using 10,000 permutations of the data set in Microsatellite Analyzer (Dieringer and Schlötterer, 2003). To examine the extent of genetic variance among sampled locations, GenAlEx v.6.503 was used to perform an analysis of molecular variance (AMOVA). Statistical significance was

TABLE 2 | Genetic Diversity Summary Statistics for Nassau Grouper where n, number of individuals; Na, number of effective alleles; A_R, allelic richness; PA_R, private allelic richness; A_S, size range of alleles; H_O, observed heterozygosity; H_E, expected heterozygosity; HWE, probability of conforming to Hardy-Weinberg equilibrium, F_{IS}, inbreeding coefficient.

Location	Est416	Est340	Est33a	Est338	EACD08	Est420	Est92	Est360	EACD02	Est262	Est290	Est376	Est267	Est265	Mean
BIMINI															
n	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Na	2	2	2	1	1	2	1	2	2	2	1	2	1	2	1.64
A_R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PA_R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A_S	142–154	232–248	133–137	175–175	130–130	199–207	184–184	174–182	269–273	264–284	330–330	181–185	207–207	177–189	–
H_O	1.000	1.000	1.000	0.000	0.000	1.000	0.000	1.000	1.000	1.000	0.000	1.000	0.000	1.000	0.643
H_E	0.500	0.500	0.500	0.000	0.000	0.500	0.000	0.500	0.500	0.500	0.000	0.500	0.000	0.500	0.321
HWE	0.317	0.317	0.317	–	–	0.317	–	0.317	0.317	0.317	–	0.317	–	0.317	0.317
F_{IS}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GRAND BAHAMA															
n	5	4	5	4	5	5	5	5	5	5	5	5	5	5	4.86
Na	7	7	3	6	6	5	8	4	7	7	6	6	3	6	5.79
A_R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PA_R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A_S	122–154	194–252	133–145	169–211	124–158	195–227	172–204	162–190	253–297	268–292	326–354	169–193	183–207	161–193	–
H_O	1.000	0.750	0.800	1.000	0.800	0.800	1.000	0.400	1.000	0.800	0.800	0.800	0.400	0.600	0.782
H_E	0.820	0.844	0.620	0.813	0.800	0.680	0.860	0.580	0.840	0.840	0.760	0.800	0.560	0.820	0.760
HWE	0.628	0.293	0.769	0.526	0.577	0.832	0.628	0.125	0.680	0.371	0.735	0.534	0.135	0.290	0.509
F_{IS}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ABACO															
n	69	65	73	69	71	70	75	74	74	72	66	75	66	75	71
Na	14	27	11	19	14	13	19	15	27	17	15	18	18	20	17.64
A_R	7.0624	9.2431	5.8978	8.187	6.2123	6.0966	8.348	6.1263	9.23	7.1683	7.4739	8.0021	7.2453	8.0402	7.45
PA_R	0.3015	1.7745	0.9312	0.7405	0.8015	0.5714	0.5111	0.3366	0.9587	0.3663	0.3745	0.8526	1.0652	0.8657	0.75
A_S	130–162	188–268	125–157	167–247	122–172	195–243	154–228	134–194	245–341	254–304	326–366	161–241	183–255	149–229	–
H_O	0.884	0.800	0.699	0.725	0.690	0.814	0.853	0.770	0.838	0.833	0.758	0.827	0.742	0.720	0.782
H_E	0.863	0.918	0.801	0.894	0.817	0.805	0.900	0.811	0.919	0.856	0.875	0.896	0.859	0.892	0.865
HWE	0.277	***	***	***	***	***	***	***	***	***	***	***	***	***	0.02*
F_{IS}	−0.021	0.133	0.131	0.193	0.158	−0.008	0.055	0.053	0.091	0.030	0.138	0.080	0.139	0.196	0.098
BERRY ISLANDS															
n	8	8	8	8	8	8	8	7	8	8	8	8	8	8	7.93
Na	6	8	5	7	6	6	11	8	10	11	8	8	7	7	7.71
A_R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PA_R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A_S	142–164	208–256	125–145	179–211	122–146	195–215	158–204	162–240	263–311	254–288	326–360	169–219	191–219	161–189	–
H_O	0.875	0.750	0.625	0.750	0.625	0.625	1.000	0.857	0.625	0.875	0.875	0.875	0.875	1.000	0.802
H_E	0.805	0.836	0.719	0.813	0.805	0.813	0.875	0.847	0.883	0.883	0.820	0.852	0.828	0.820	0.828
HWE	0.400	0.573	0.035*	0.655	0.246	0.559	0.856	0.404	0.163	0.437	0.929	0.573	0.663	0.663	0.511
F_{IS}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
ANDROS															
n	50	46	50	46	50	48	49	49	50	49	48	50	48	50	48.79
Na	15	16	11	15	13	12	20	19	28	18	15	11	12	12	15.50
A_R	8.2648	8.3842	5.4389	8.223	6.5583	6.2929	9.2895	8.369	10.0372	7.8629	7.9736	6.7765	6.87	7.5245	7.70
PA_R	1.0483	0.3808	0.489	0.5109	0.7261	0.4007	1.07	1.0333	1.1876	0.5061	0.4238	0.1729	0.612	0.2331	0.63
A_S	130–168	208–260	121–149	169–219	122–166	181–223	150–240	142–198	245–325	250–300	322–362	161–201	187–225	157–225	–
H_O	0.900	0.848	0.720	0.826	0.840	0.792	0.918	0.755	0.920	0.898	0.854	0.800	0.813	0.900	0.842
H_E	0.898	0.903	0.754	0.894	0.830	0.814	0.918	0.892	0.931	0.878	0.874	0.855	0.844	0.884	0.869
HWE	0.541	*	***	0.040	***	0.028	**	***	***	***	0.218	0.967	0.932	0.267	0.216
F_{IS}	0.002	0.066	0.050	0.082	−0.007	0.033	0.005	0.158	0.017	−0.017	0.028	0.069	0.043	−0.014	0.037

(Continued)

TABLE 2 | Continued

Location	Est416	Est340	Est33a	Est338	EACD08	Est420	Est92	Est360	EACD02	Est262	Est290	Est376	Est267	Est265	Mean
NEW PROVIDENCE															
n	10	9	10	8	10	10	10	9	10	9	9	10	7	9	9.29
Na	9	7	8	11	6	9	8	7	9	9	9	9	7	10	8.43
A_R	7.7807	6.3186	6.639	9.9833	5.3649	7.1961	6.8627	6.616	7.6798	8.1373	7.6225	7.7807	7	8.766	7.41
PA_R	0.0365	0.0037	0.9774	1.6816	0.1605	0.731	0.0195	0.1489	0.2117	0.6234	0.2094	0.0057	0.0315	1.5074	0.45
A_S	130–158	216–252	125–149	175–239	130–154	193–219	164–200	162–190	253–313	254–300	322–354	169–201	187–219	161–201	–
H_O	0.800	0.889	0.600	1.000	0.800	0.900	1.000	1.000	0.900	1.000	0.778	0.900	0.857	0.778	0.872
H_E	0.850	0.796	0.770	0.875	0.770	0.820	0.810	0.809	0.855	0.852	0.790	0.850	0.837	0.870	0.825
HWE	0.583	0.171	0.096	0.801	0.375	0.075	0.500	0.490	0.102	0.511	0.393	0.441	0.252	0.273	0.362
F_{IS}	0.084	−0.088	0.245	−0.111	−0.013	−0.072	−0.210	−0.209	−0.027	−0.146	0.044	−0.033	0.013	0.135	−0.028
ELEUTHERA															
n	62	61	63	59	62	62	63	62	63	53	61	63	59	62	61.07
Na	21	21	9	15	10	11	14	12	17	13	11	10	11	12	12.71
A_R	6.402	8.7477	5.1088	8.145	6.0237	5.9114	7.9528	6.1275	8.7966	6.6986	6.7658	7.0535	6.6449	7.5472	6.99
PA_R	0.2101	0.8612	0.2555	0.2999	0.1746	0.3208	0.2235	0.358	0.3313	0.1679	0.1115	0.0407	0.0519	0.1978	0.26
A_S	130–162	188–276	117–145	167–245	122–166	191–221	158–212	162–206	253–317	254–308	322–366	169–201	187–227	157–221	–
H_O	0.855	0.885	0.746	0.898	0.772	0.774	0.905	0.871	0.905	0.774	0.738	0.841	0.729	0.903	0.826
H_E	0.844	0.909	0.736	0.896	0.828	0.812	0.895	0.817	0.912	0.836	0.845	0.865	0.851	0.883	0.852
HWE	***	***	***	0.078	***	***	0.287	***	0.690	***	0.163	0.781	***	***	0.140
F_{IS}	−0.009	0.030	−0.010	0.002	0.108	0.051	−0.007	−0.062	0.012	0.079	0.131	0.032	0.148	−0.019	0.035
CAT ISLAND															
n	7	6	7	6	7	6	7	6	7	6	7	7	7	7	6.64
Na	8	7	5	11	6	4	9	8	9	8	9	7	8	8	7.64
A_R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PA_R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A_S	134–158	208–260	125–145	167–215	122–146	195–211	158–206	156–182	253–309	262–280	326–360	169–201	183–223	161–225	–
H_O	0.857	0.833	0.857	1.000	0.857	0.833	0.714	1.000	0.857	0.667	0.857	0.714	0.857	1.000	0.850
H_E	0.837	0.819	0.735	0.903	0.796	0.625	0.857	0.833	0.867	0.778	0.867	0.816	0.796	0.847	0.813
HWE	0.745	0.479	0.515	0.513	0.468	0.654	0.443	0.302	0.628	0.137	0.591	0.668	0.957	0.570	0.548
F_{IS}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
EXUMA															
n	43	43	44	43	44	44	43	42	44	41	44	44	44	44	43.36
Na	13	15	7	14	10	10	21	21	24	18	21	19	20	17	16.43
A_R	6.9368	8.2435	4.931	7.403	6.0094	5.9603	9.8469	9.1517	9.7304	9.0221	9.1757	8.0366	8.0162	8.6086	7.93
PA_R	0.389	0.3071	0.2307	0.2967	0.4916	0.15	1.396	1.8416	1.372	1.4812	1.5804	1.4885	1.6381	1.0729	0.98
A_S	122–164	208–260	125–145	171–245	118–158	195–223	140–224	138–214	249–329	248–300	320–378	161–257	185–279	149–245	–
H_O	0.884	0.953	0.773	0.791	0.632	0.932	0.907	0.738	0.750	0.829	0.864	0.841	0.795	0.886	0.827
H_E	0.855	0.893	0.760	0.862	0.803	0.801	0.930	0.914	0.926	0.911	0.907	0.876	0.878	0.907	0.873
HWE	0.814	***	***	***	***	**	**	***	***	**	***	***	***	***	0.059
F_{IS}	−0.028	−0.061	−0.012	0.088	0.213	−0.158	0.031	0.198	0.195	0.096	0.054	0.046	0.099	0.028	0.056
HAIL MARY															
n	56	57	57	55	57	57	57	57	56	46	55	57	56	57	55.71
Na	15	16	8	17	10	10	20	15	23	15	13	14	12	14	14.43
A_R	7.3025	7.584	5.2574	8.7108	5.7725	6.5192	8.513	6.9404	9.3595	7.5238	7.084	7.3963	6.8864	7.8208	7.33
PA_R	0.6544	0.1612	0.2025	0.3566	0.5137	0.1103	0.6768	0.4945	0.8047	0.5075	0.4002	0.6502	0.1984	0.2501	0.43
A_S	130–166	208–264	125–153	167–239	112–164	193–223	154–208	162–214	245–325	252–300	326–370	161–211	187–231	145–205	–
H_O	0.957	0.912	0.789	0.891	0.772	0.860	0.860	0.842	0.875	0.783	0.800	0.825	0.875	0.842	0.842
H_E	0.866	0.879	0.783	0.908	0.807	0.835	0.902	0.837	0.919	0.863	0.855	0.866	0.855	0.888	0.862
HWE	0.329	0.994	**	0.953	***	***	0.977	*	***	**	0.055	***	**	***	0.241
F_{IS}	0.015	−0.034	−0.004	0.024	0.048	−0.025	0.051	−0.001	0.195	0.099	0.068	0.052	−0.019	0.056	0.037

(Continued)

TABLE 2 | Continued

Location	Est416	Est340	Est33a	Est338	EACD08	Est420	Est92	Est360	EACD02	Est262	Est290	Est376	Est267	Est265	Mean
LONG ISLAND															
n	21	21	20	21	19	21	21	20	21	21	20	21	19	21	20.50
Na	9	15	7	11	8	11	16	13	22	16	13	11	10	11	12.36
A_R	6.7183	9.2563	5.4424	8.2464	5.7164	7.4781	9.9631	8.6753	11.2659	9.223	7.8625	7.0235	7.3546	7.1241	7.95
PA_R	0.054	1.128	0.3235	0.1778	0.2579	0.6073	1.6817	1.8309	3.0309	1.7939	1.114	0.3674	0.1033	0.0479	0.89
A_S	130–162	208–264	125–149	167–215	122–154	195–223	156–240	168–218	243–325	250–296	328–362	161–213	187–223	157–197	–
H_O	0.905	0.952	0.750	0.762	0.789	0.857	0.667	0.700	0.810	0.571	0.750	0.714	0.789	0.810	0.773
H_E	0.845	0.909	0.778	0.893	0.788	0.868	0.923	0.896	0.943	0.904	0.870	0.832	0.852	0.840	0.867
HWE	0.668	0.169	0.137	0.316	**	0.658	**	0.087	0.049	***	0.092	0.618	0.117	0.894	0.273
F_{IS}	−0.059	−0.035	0.048	0.159	0.012	0.025	0.289	0.231	0.154	0.378	0.150	0.153	0.086	0.048	0.117
ACKLINS															
n	5	5	5	5	5	5	5	5	5	5	5	5	5	5	5
Na	6	7	4	5	6	5	7	4	7	5	6	6	6	6	5.71
A_R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PA_R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A_S	130–166	224–260	125–141	175–203	122–164	195–211	168–200	174–188	253–313	260–292	322–358	169–201	187–215	161–193	–
H_O	0.800	0.800	0.600	0.800	1.000	0.800	0.800	0.800	1.000	0.400	0.800	1.000	1.000	1.000	0.829
H_E	0.700	0.840	0.640	0.760	0.800	0.720	0.820	0.660	0.820	0.760	0.700	0.800	0.760	0.760	0.753
HWE	0.694	0.371	0.477	0.132	0.704	0.794	0.486	0.890	0.247	0.113	0.694	0.704	0.890	0.890	0.578
F_{IS}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
RAGGED ISLAND															
n	7	7	6	7	6	7	7	6	7	6	6	7	6	7	6.57
Na	7	7	4	6	4	6	9	7	10	10	6	8	7	6	6.93
A_R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
PA_R	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
A_S	130–158	216–252	125–141	179–211	130–142	195–219	160–208	160–182	255–301	254–286	328–350	161–197	179–223	161–193	–
H_O	0.714	0.571	0.833	0.714	0.500	0.714	0.857	0.833	0.857	1.000	1.000	0.857	0.833	0.571	0.776
H_E	0.827	0.816	0.736	0.816	0.625	0.755	0.878	0.819	0.888	0.889	0.792	0.857	0.764	0.786	0.803
HWE	0.197	0.447	0.238	0.585	0.722	0.348	0.133	0.262	0.126	0.472	0.263	0.527	0.588	0.306	0.372
F_{IS}	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GREAT INAGUA															
n	46	46	46	46	46	46	46	46	46	44	45	46	46	46	45.79
Na	17	19	12	17	10	14	14	13	18	13	13	13	11	15	14.21
A_R	7.2717	8.839	6.7449	8.373	6.29	6.8761	8.2756	6.037	8.554	7.7882	7.4392	7.2136	7.3065	8.1493	7.51
PA_R	1.029	0.6896	1.0002	0.9157	0.736	0.4924	0.3119	0.4496	0.6181	0.1497	0.331	0.3052	0.1842	0.5049	0.55
A_S	118–166	208–260	109–141	171–217	122–154	191–223	158–204	162–214	245–321	254–300	322–374	161–219	187–227	153–233	–
H_O	0.935	0.913	0.801	0.891	0.739	0.783	0.870	0.848	0.870	0.841	0.867	0.804	0.870	0.891	0.852
H_E	0.850	0.912	0.847	0.893	0.830	0.822	0.898	0.791	0.901	0.877	0.875	0.864	0.865	0.894	0.866
HWE	***	**	***	***	***	**	0.084	***	*	***	0.824	0.957	0.272	0.828	0.213
F_{IS}	−0.095	0.004	0.055	0.007	0.115	0.054	0.037	−0.066	0.040	0.047	0.015	0.074	0.001	0.009	0.021
UNKNOWN															
n	51	50	54	53	52	55	54	51	55	51	48	55	47	54	52.14
Na	11	16	8	15	10	13	17	16	24	16	17	11	10	11	13.93
A_R	6.9046	8.344	4.8605	8.0518	5.4128	6.2877	8.5563	7.1816	9.0838	8.0143	8.0323	7.0614	6.7997	7.4854	7.29
PA_R	0.1451	0.4164	0.2241	0.1415	0.3252	0.5558	0.6031	0.5562	1.0565	0.3876	0.5118	0.2071	0.0638	0.24	0.39
A_S	130–168	184–260	113–145	167–215	120–158	185–223	158–232	130–198	245–317	254–296	322–358	169–205	187–227	157–209	–
H_O	0.824	0.960	0.667	0.849	0.500	0.909	0.722	0.725	0.891	0.804	0.875	0.873	0.745	0.796	0.796
H_E	0.862	0.898	0.736	0.888	0.794	0.815	0.904	0.850	0.912	0.888	0.882	0.866	0.854	0.879	0.859
HWE	0.134	***	***	0.071	***	***	***	***	**	***	***	0.057	***	***	0.019*
F_{IS}	0.050	−0.064	0.099	0.048	0.374	−0.111	0.205	0.151	0.028	0.100	0.013	−0.003	0.134	0.099	0.080

*Significant HWE values are denoted as follows: *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.*

assessed using 999 permutations of the data. Principal coordinate analysis (PoCA) plots were generated from codominant genetic distance matrices for all samples ($n = 454$) initially and then for locations with sample sizes of ≥10 per location ($n = 421$) in GenAlEx v.6.503. A discriminate analysis of principal components (DAPC) was also constructed using the *adegenet* package in R to expose any fine-scale genetic partitioning (Jombart et al., 2010).

To visualize genetic population structure, the admixture ancestry model using correlated allele frequencies in STRUCTURE v.2.3.4 (Pritchard et al., 2000) was implemented with a pre- and post-burn-in of 75,000 and 150,000 Monte Carlo Markov Chain iterations respectively over 10 independent runs for inferred populations, *K,* ranging from 1 to 17. STRUCTURE analysis was re-run using a pre- and post-burn-in of 250,000 and 1,000,000 MCMCs respectively for *K* 1–10. The most likely partition of the dataset for both STRUCTURE runs was determined using the delta *K* (ΔK) statistic (Supplementary Figures 1, 2; Evanno et al., 2005) and was calculated using the web-based Structure Harvester v. 0.6.94 programme (Earl and vonHoldt, 2012). The PopHelper web application v.1.0.10 (with a ΔK of 2) was used to create a Structure plot of aligned and merged repeats for the 10 independent runs of *K* 1–10. Because marine species often show weak patterns of genetic differentiation, the locprior model (Hubisz et al., 2009) was also run with parameters identical to those used for the admixture model for *K* 1–10. Because the ΔK was the same for both models, only the results of the more conservative admixture model (which assumes no prior information about structure) are reported.

Finally, an assessment of whether genetic structure could be attributed to isolation-by-distance was performed using a Mantel test in GenAlEx v.6.503. Matrices of unbiased pairwise genetic distances $F_{ST}/(1-F_{ST})$ were compared with the logarithm of geographic distances between sites (km). Google Earth Pro was used to estimate the shortest straight line distance between islands. Significance was based on 999 permutations of the data.

Effective Population Size and Population Bottlenecks

Estimates of contemporary effective population size, N_e based on linkage disequilibrium were assessed using the random mating model with minimum allele frequencies of 0.05, 0.02, 0.01, and 0.005, as implemented in LDNE v.1.31 (Waples and Do, 2008). LDNE analyses were performed using sub-adult and adult fish from Eleuthera and Exuma and for the entire dataset. Additionally, we used STRUCTURE outputs for $\Delta K = 2$ to assign individuals to the most likely genetic cluster and re-analyzed contemporary N_e using these two populations. M-ratio was used to test whether anthropogenic activities (e.g., overfishing) have led to declines in population size of Nassau grouper throughout the archipelago and within an active FSA. We calculated the M-ratio, the ratio of the number of microsatellite alleles compared to allele size range (Garza and Williamson, 2001). Critical M (M_c) values were computed using a pre-bottleneck N_e ranging from 10,000 to 15,000; corresponding to theta (θ) values between 5.6 and 120 and a mutation rate of $\mu = 5 \times 10^{-4}$. We also tested mutation rates of 5.57×10^{-4}, 2.0×10^{-3}, and 1.5×10^{-4} derived from Common carp (*Cyprinus carpio*, Yue et al., 2007), Broadnosed pipefish (*Syngnathus typhle*, Jones et al., 1999) and Zebrafish (*Danio rerio*, Shimoda et al., 1999). The proportion of stepwise mutations was set to 0.78 and the mean size of non-stepwise mutation (δ_g) to 3.1 following the suggestions of Peery et al. (2012).

Temporal changes in N_e were analyzed using the *VarEff* package in R to determine when historical population declines occurred (Nikolic and Chevalet, 2014). The *VarEff* model uses the coalescent method and approximate likelihoods to derive posterior distributions of N_e over a specified past generation time. Initially, this analysis was performed assuming a two-phase model, T, with a commonly used microsatellite mutation rate, $\mu = 5 \times 10^{-4}$ (Estoup et al., 2002), and burn-in of 10,000 over the past 1,000 generations. Additional parameters included setting the number batch to 50,000, length and space batch to 10, with an acceptance rate of 0.25 and diagonale of 0.5, following recommendations from Nikolic and Chevalet (2014). The model was also run using mutation rates of 5.57×10^{-4} (*C. carpio*), 2.0×10^{-3} (*S. typhle*) and 1.5×10^{-4} (*D. rerio*) respectively, under the same parameters. The procedure was re-run modifying the number of times N_e changed (JMAX 2 and 3) and assumed prior values of N_e (NBAR 10,000 and 15,000) for each of the mutation rates listed above (Supplementary Figures 3–6).

RESULTS

Quality Control of Genotypes

Following the removal of 10 individuals with missing data for four or more loci, genotypes from 454 Nassau grouper were retained for analysis. Evidence of null alleles and homozygote excess or heterozygote deficiencies were detected in eight loci, but these were inconsistent across sample locations (Supplementary Table 3). Three occurrences of stuttering and potential scoring error were detected for loci EACB6 and Est360. However, no large allele dropout was detected, and stuttering and scoring error were negligible across populations (Supplementary Table 3). Tests for linkage disequilibrium revealed associations between 10 pairs of loci for fish collected from unknown locations within The Bahamas and Abaco, Eleuthera and Exuma. Samples at three locations, Abaco, Exuma and Long Island, showed possible deviations from Hardy Weinberg equilibrium. Locus EACB6 exhibited significant deviations from HWE after FDR correction across ≥50% of populations and 10 instances of homozygote excess, which can be indicative of null alleles. Accordingly, genotypes for this locus were excluded from subsequent analyses.

Genetic Diversity

Microsatellite loci were highly polymorphic, with the number of alleles per locus ranging from 21 (Est33a) to 42 (EACD02; Supplementary Table 5). A total of 394 alleles were detected, averaging 28.14 across all 14 loci. Measures of genetic diversity and allele size range varied among loci and across sample locations (**Table 2**, Supplementary Tables 4, 5). Gene diversity

by sampling year and life stage ranged from $H_E = 0.746–0.873$ and from 0.838 to 0.877 respectively (Supplementary Table 4). Estimates of allelic richness were variable, ranging from $A_R = 4.4–7.84$ (Supplementary Table 4). Inbreeding coefficients also varied by sampling year and life stage, with higher F_{IS} values observed for sub-adults vs. adults (Supplementary Table 4). Allelic richness for the pooled dataset (adjusted for sample size) was similar among locations, with values ranging from 6.99 to 7.95 (**Table 2**). Private allelic richness varied from 0.26 to 0.89. Mean allelic richness was highest in Long Island (7.95) and lowest in Eleuthera (6.99). Private alleles were identified in all locations, with more private alleles present in Exuma (mean PA_R of 0.98) specimens (**Table 2**). Heterozygosity, H_E was generally high across the sampling area (ranging from 0.753 to 0.873), with the exception of Bimini (0.321), which contained the smallest samples size (**Table 2**). Mean H_O was lowest in Bimini (0.643) and highest in New Providence (0.872). Long Island had the highest mean inbreeding coefficient, ($F_{IS} = 0.117$) and New Providence had the lowest ($F_{IS} = -0.028$). Kruskal-Wallis tests showed no significant differences in H_E, H_O, A_R, and PA_R among locations ($p > 0.05$).

Genetic Differentiation and Demographic Analyses

PCoA, DAPC, and STRUCTURE analyses revealed no distinct clustering or definitive geographic structure in the genetic composition of Bahamian Nassau grouper when examined

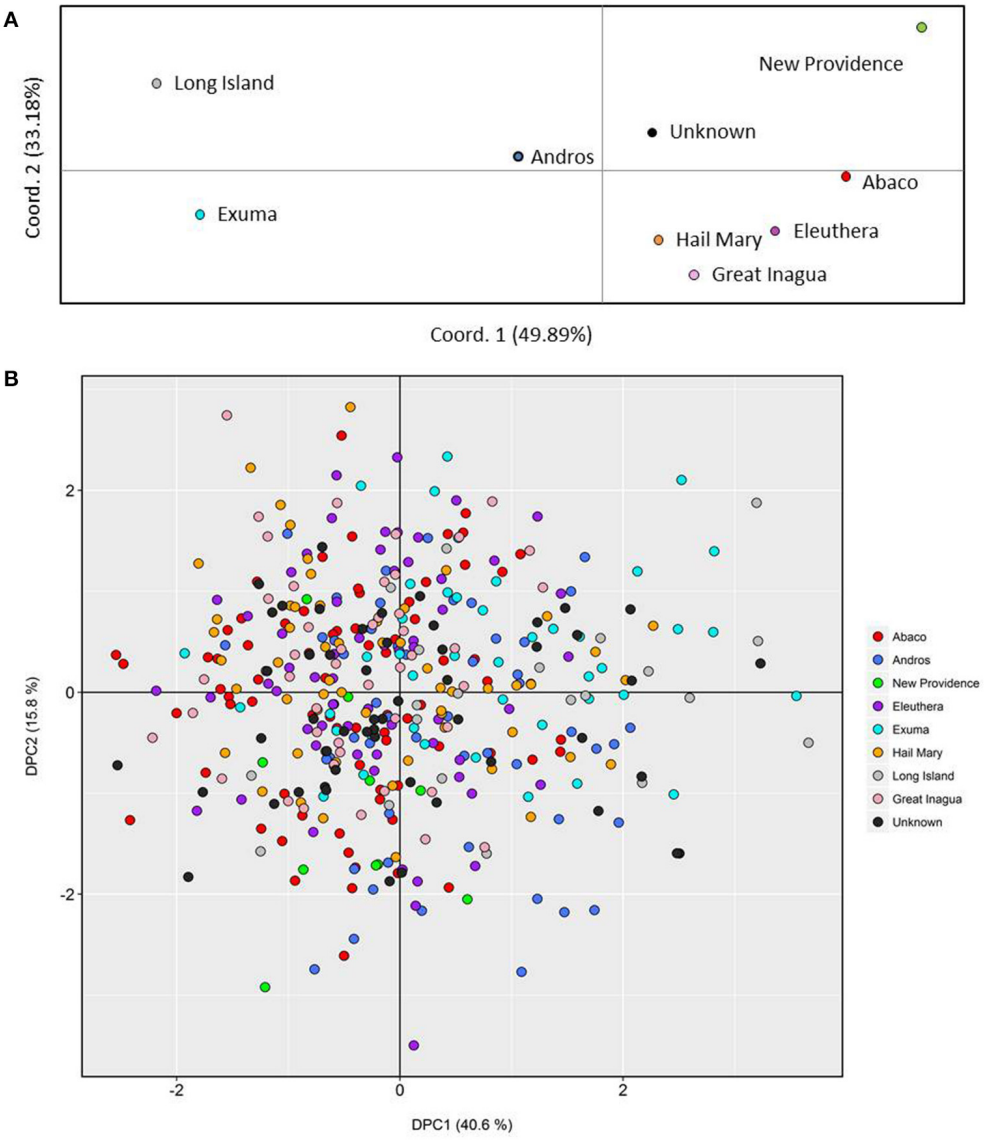


FIGURE 2 | Genetic clustering of Nassau grouper based upon 421 genotypes and inferred from **(A)** Principal Coordinates Analysis (PCoA) by location and **(B)** individual based Discriminate Analysis of Principal Components (DAPC). The per cent variation explained by each axis is presented within brackets for both the PCoA and DAPC.

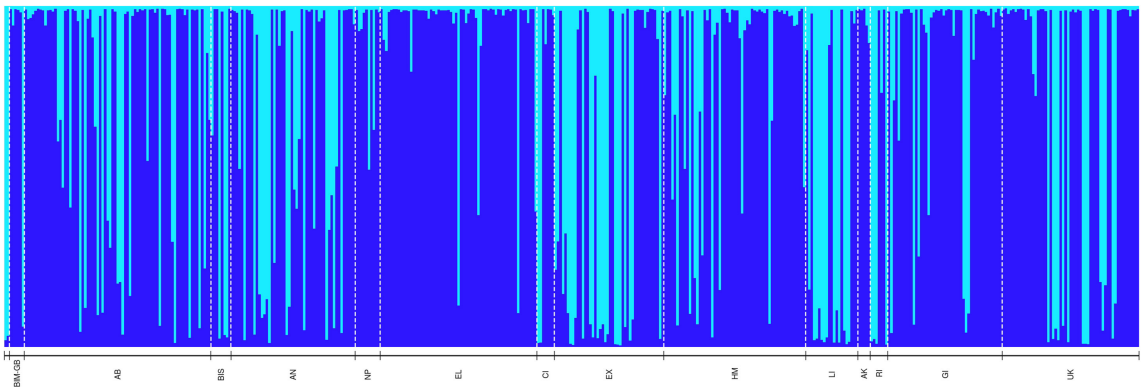


FIGURE 3 | Bayesian-based Structure analysis depicting genetic clustering ($K = 2$) of 454 Nassau grouper sampled organized geographically from north to south (left-right) throughout The Bahamas ($n = 454$). Codes correspond to the following sampling locations: BIM-GB includes pooled samples from Bimini ($n = 1$) and GB = Grand Bahama ($n = 5$), AB, Abaco; BIS, Berry Islands; AN, Andros; NP, New Providence; EL, Eleuthera; CI, Cat Island; EX, Exuma; HM, Hail Mary FSA; LI, Long Island; AK, Acklins; RI, Ragged Island; GI, Great Inagua; UK, Unknown.

TABLE 3 | Results of Nassau grouper population differentiation within The Bahamas based on pairwise F_{ST} values.

Location	Abaco	Andros	New providence	Eleuthera	Exuma	Hail mary	Long island	Great inagua	Unknown
Abaco	0	0.0022	0.0020	−0.0002	0.0067	−0.0011	0.0095	−0.0015	0.0024
Andros	0.0022	0	0.0035	0.0022	0.0014	0.0013	0.0048	0.0018	0.0001
New Providence	0.0020	0.0035	0	0.0035	0.0099	0.0035	0.0079	0.0057	0.0032
Eleuthera	−0.0002	0.0022	0.0035	0	0.0045	−0.0004	0.0119	0.0006	0.0017
Exuma	**0.0067**	0.0014	0.0099	**0.0045**	0	0.0035	0.0022	0.0052	0.0043
Hail Mary	−0.0011	0.0013	0.0035	−0.0004	0.0035	0	0.0072	−0.0010	0.0006
Long Island	**0.0095**	0.0048	0.0079	**0.0119**	0.0022	**0.0072**	0	0.0104	0.0063
Great Inagua	−0.0015	0.0018	0.0057	0.0006	**0.0052**	−0.0010	**0.0104**	0	0.0009
Unknown	0.0024	0.0001	0.0032	0.0017	**0.0043**	0.0006	0.0063	0.0009	0

Bonferroni corrected F_{ST} values are beneath the diagonal, with significant values in bold.
Uncorrected F_{ST} values appear above the diagonal.

by island or individual (**Figures 2**, **3**). However, there was a low but significant difference in the overall F_{ST} value across samples and loci (Global $F_{ST} = 0.00236$, $p = 0.0001$; **Table 3**). Genetic differentiation based on pairwise F_{ST} values ranged from −0.00097 to 0.01194 and was greatest between Eleuthera and Long Island. Pairwise F_{ST} values were significant between only 8 population pairs (**Table 3**). The Mantel test revealed no significant isolation-by-distance (rxy = −0.208, $p = 0.255$; **Figure 4**). AMOVA results indicated that most genetic variability, 90%, occurs within individuals, with 10% among individuals (Supplementary Table 6).

Effective Population Size and Population Bottlenecks

Contemporary linkage disequilibrium based estimates of N_e varied among locations and in association with the allele frequency used (**Table 4**, Supplementary Table 7). N_e also varied by sampling year, life stage and population (Supplementary Table 7). Values based on a critical allele frequency of 0.02 are reported (**Table 4**) following recommendations by Waples and Do (2010). N_e was lowest for New Providence (−888.3), but this was likely due to the small sample size. To investigate this, we randomly sub-sampled data ($n = 10$) from Abaco, Eleuthera and Andros respectively and re-ran LDNE analysis, which also produced negative N_e estimates (Supplementary Table 8). Excluding New Providence, N_e estimates were lowest for Abaco (∼375) and highest for Andros (∼2,978).

The *VarEff* modeling approach using $\mu = 5 \times 10^{-4}$ revealed historically very large and stable effective population sizes for Nassau grouper within The Bahamas (**Figure 5**). The upper estimates of median N_e ranged from ∼111,000 in Long Island to ∼315,000 in Andros. N_e estimates for The Bahamas peaked at ∼227,000 and began to gradually decline around 400 generations ago. Similar patterns of population decline were identified for all islands in The Bahamas and Hail Mary around the same period, with a severe decline occurring within the last 150 generations (**Figure 5**). The upper estimates for Nassau grouper within the last 100 generations were variable, ranging from ∼135,742 in Great Inagua to 5,024 Long Island (**Figure 5**). Lower and upper contemporary N_e estimates during the last 50 generations varied from ∼830 to

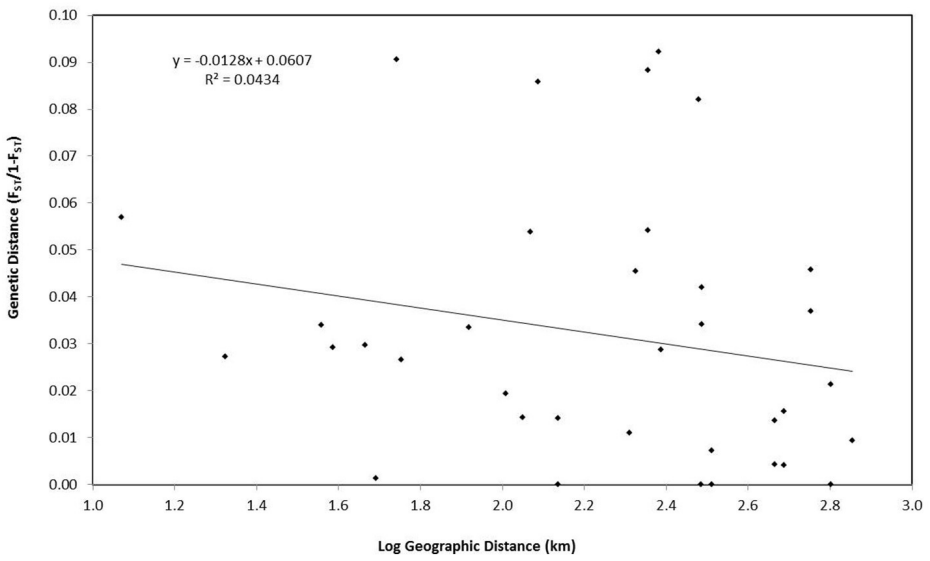


FIGURE 4 | Correlation between genetic distance and geographic distance for Nassau grouper.

TABLE 4 | Summary of effective population size, N_e per location with 95% confidence intervals, and M-ratio results.

Location	N_g	LDNE N_e (95% CI)	M-ratio	M_c for $\Theta = 20$	M_c for $\Theta = 30$
Abaco	75	374.7 (239.4–809.9)	**0.564**	0.601	0.596
Andros	50	2,978.2 (481.1–∞)	0.601	0.565	0.555
New Providence	10	−888.3 (44.0–∞)	0.466	0.359	0.328
Eleuthera	63	619.6 (286.8–∞)	**0.512**	0.587	0.580
Exuma	44	418.3 (226.1–2216.1)	**0.530**	0.548	0.538
Hail Mary	57	2,027.7 (435.2–∞)	**0.560**	0.576	0.568
Long Island	21	779.4 (158.1–∞)	0.534	0.463	0.438
Great Inagua	46	1,637.0 (381.0–∞)	0.568	0.555	0.542

M-ratio data for pre-bottlenecks N_e values of 10,000 (M_c for $\Theta = 20$) and 15,000 (M_c for $\Theta = 30$) are reported using a mutation rate of 5×10^{-4}.
N_g denotes the number of genotyped individuals included in the analyses for each location. Locations which experience population bottlenecks are in bold.

1,514 in Great Inagua and ∼2,672 to 4,539 in Exuma. The upper estimate for The Bahamas during this timeframe was ∼2,016 (**Figure 5**).

Tests using faster and slower mutation rates shifted the timing of genetic bottlenecks (Supplementary Figures 3–6). Under a faster rate of mutation ($\mu = 2 \times 10^{-3}$), bottlenecks would have occurred within the last 50 generations, whereas under the slower mutation rate ($\mu = 1.5 \times 10^{-4}$), declines occurred around 300–400 generations ago (Supplementary Figure 6). All model simulations consistently revealed a pronounced decline in Nassau grouper within the past 400 generations for all islands (Supplementary Figures 3–6). The only exceptions were Exuma and Long Island, which under the slower mutation rate, showed population declines within the last 450 generations (Supplementary Figure 5).

Dating when bottlenecks occurred varied in accordance with the generation time used. We explored this using generation times ranging from 4 to 9 years. These times encompass the earliest age of sexual maturity, 4 years (Sadovy and Colin, 1995), the suggested generation time calculated for an unexploited Nassau grouper FSA, 9 years (Sadovy and Eklund, 1999), and the approximate age at which Bahamian Nassau grouper undertake their first spawning migration, 7 years (Dahlgren et al., 2016a). Comparisons of historical vs. contemporary derived *VarEff* N_e estimates for The Bahamas indicate a dramatic reduction in N_e over the last 400 generations, which would have occurred between ∼1,600–3,600 years ago (corresponding to generation times of 4 and 9 years respectively). M-ratio estimates also varied in relation to the mutation rate used. Generally, under the fastest mutation rate, no bottlenecks were detected, however, under more realistic mutation rates, M-ratio values were below critical M values for both pre-bottleneck thetas (Supplementary Table 9). Overall, *VarEff* outputs were consistent with M-ratio analyses, showing historic bottlenecks on several islands and throughout The Bahamas (**Table 4**, **Figure 5**).

DISCUSSION

As a species at risk of extinction, developing and applying science-based conservation management policies at the

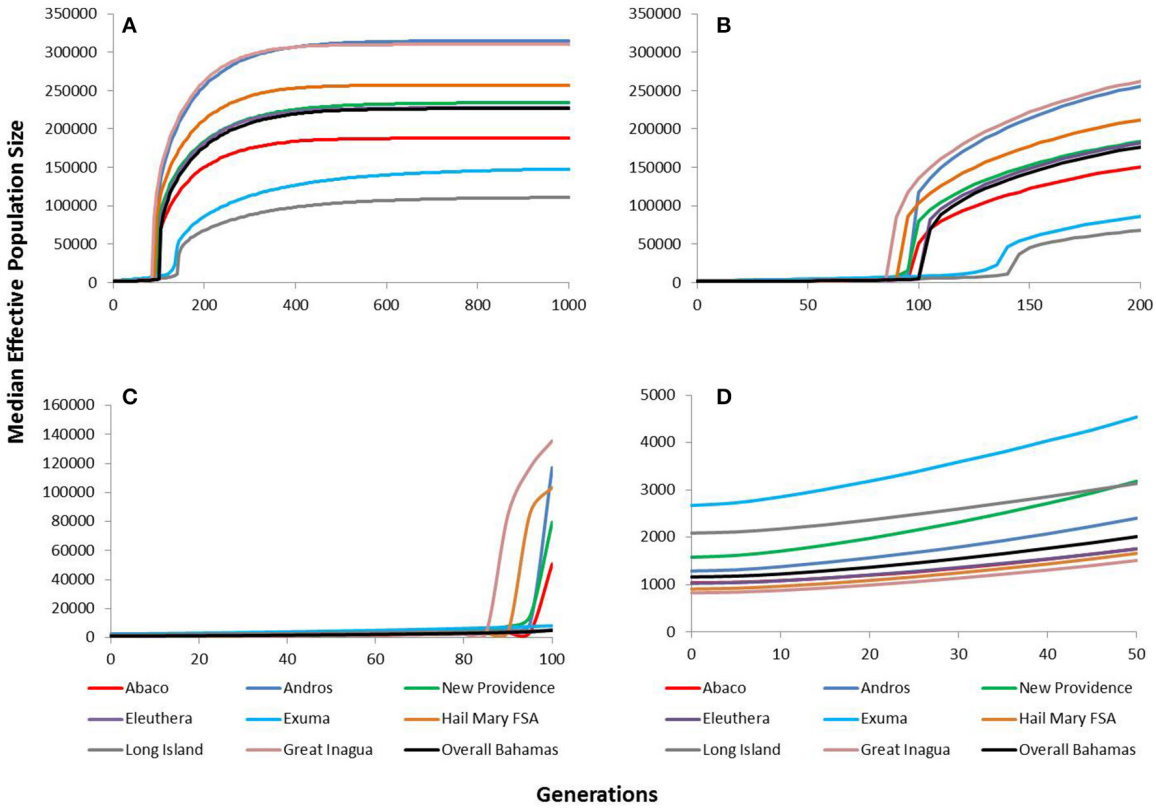


FIGURE 5 | *VarEff* estimates of temporal changes in the effective population sizes, N_e of Nassau grouper from Abaco (red), Andros (blue), New Providence (green), Eleuthera (purple), Exuma (blue), Hail Mary FSA (tan), Long Island (gray), Great Inagua (pink) and overall Bahamas (black) for **(A)** the past 1,000 generations, **(B)** the past 200 generations, **(C)** the past 100 generations, and **(D)** the past 50 generations.

appropriate scale are critical to facilitate recovery of Nassau grouper. From a conservation perspective, the preservation of genetic variation is an important component for mitigating biodiversity loss, highlighting the necessity for research in this area. We aimed to delineate population structure and establish N_e estimates for Nassau grouper throughout the Bahamian archipelago to support current conservation efforts. The highly polymorphic nature of the species-specific microsatellites employed for this study support their utilization for assessing genetic variability and population subdivision.

Genetic Diversity, Differentiation, and Demographic Analyses

Results from microsatellite analysis of genotyped Nassau grouper revealed weak genetic differentiation, lacking definitive geographical population structure. Patterns of weak or no genetic subdivision at varying spatial and temporal scales using microsatellites and other molecular markers have been reported for a number of Epinephelids, e.g., Camouflage grouper, *E. polyphekadion* (Rhodes et al., 2003); Hawaiian grouper, *E. quernus* (Rivera et al., 2004); Red grouper, *E. morio* (Zatcoff et al., 2004); Dusky grouper, *E. marginatus* (Maggio et al., 2006; Schunter et al., 2011; Buchholz-Sørensen and Vella, 2016) and Goliath grouper, *E. itajara* (Silva-Oliveira et al., 2008). We expected to see a stronger signal of genetic differentiation separating the northern and southern Bahamas given that the Exumas were purported to present a barrier to gene flow (Jackson A. M. et al., 2014). However, Nassau grouper sampled throughout the archipelago were overlapping in DAPC analysis and no geographical clustering was apparent in either PCoA or Bayesian-based Structure analyses. Moreover, a closer examination of fish sampled north to south across (∼200 km) in the Exuma chain, revealed considerable genetic mixing, which is inconsistent with the hypothesis of reduced gene flow. In contrast, low estimates of genetic differentiation and similarly high levels of genetic diversity suggest moderate to extensive gene flow or connectivity throughout The Bahamas.

There are several plausible explanations for the patterns of genetic variation observed. Firstly, pelagic larval dispersal (PLD) and recruitment success are affected by many factors including interspecific competition, predation, larval behavior, oceanographic processes, environmental conditions (e.g., water temperature) and habitat suitability (Colin, 1992, 2012; Shenker et al., 1993; Choat, 2012; Hamner and Largier, 2012; Kough et al., 2016). The stochastic nature of these biotic and abiotic factors helps to determine population structure and demographic connectivity of adults. For example, in bonefishes (*Albula* spp.),

the convergence of larval dispersal and ecological processes has been shown to influence genetic population structuring (Wallace, 2015; Wallace and Tringali, 2016). Nassau grouper have a protracted PLD of up to 50 days, with a mean of 42 days (Tucker and Woodard, 1991; Colin et al., 1997). In the Caribbean, *in situ* monitoring of sub-surface currents along with modeled trajectories of ocean currents and larval dispersal of Nassau grouper have shown varied recruitment patterns including advection from spawning sites and retention via eddies (Colin, 1992; Heppell et al., 2009, 2011). However, behavioral traits and ecology of larval Nassau grouper in tandem with local oceanographic-driven current patterns have not been explicitly investigated for The Bahamas.

Secondly, adult Nassau grouper can undertake long migrations (>300 km) to participate in annual transient FSAs (Bolden, 2000; Domeier, 2012; Dahlgren et al., 2016a; Stump et al., 2017). Telemetry studies have demonstrated that adults exhibit philopatry to specific FSAs (Starr et al., 2007; Dahlgren et al., 2016a). It is likely therefore, that the population dynamics of Nassau grouper is influenced by both adult migration to and from FSAs via continental shelf margins (Bolden, 2000; Stump et al., 2017), FSA site fidelity (Sala et al., 2001; Starr et al., 2007; Dahlgren et al., 2016a), larval dispersal (Colin, 1992; Shenker et al., 1993) and ocean currents (Colin et al., 1997; Paris, 2009; Paris et al., 2013). Ocean circulation patterns in The Bahamas show predominately northerly flowing currents (**Figure 1**); however, these patterns are not static and exhibit considerable complexity (Gunn and Watt, 1982; Colin, 1992, 1995). Strong winds and cross-continental shelf currents driven by winter cold fronts have been shown to facilitate larval dispersal from FSAs to recruitment habitats (e.g., Shenker et al., 1993; Colin, 1995; but see Colin et al., 1997). Additionally, mesoscale gyres have been documented in the Exuma Sound, enabling entrainment of larvae through to recruitment stage within this area (Colin, 1995). Collectively, the processes of adult migration and larval dispersal have the potential to increase gene flow, thus contributing to the apparent maintenance of genetic diversity despite ongoing anthropogenic pressures (e.g., FSA fishing).

However, the ability to detect genetic differentiation can be masked by high levels of polymorphism, genetic diversity and large N_e, which can act to retard mutation-drift equilibrium (O'Reilly et al., 2004; Hellberg, 2009). In this study, both patterns of genetic partitioning ($K = 2$) and a high degree of genetic diversity were found. The measures of genetic diversity reported here ($H_E = 0.753$–0.873) using neutral microsatellite markers are comparable to those found for Nassau grouper in the Caribbean ($H_E = 0.608$–0.966, Jackson A. M. et al., 2014; $H_E = 0.32$–0.91, Bernard et al., 2016) and to other grouper species ($H_E = 0.703$–0.762, Schunter et al., 2011; $H_E = 0.04$–0.886, Buchholz-Sørensen and Vella, 2016). However, given the extent of overfishing and other anthropogenic activities, which have been shown to collapse FSAs (Aguilar-Perera, 2006; Sadovy De Mitcheson et al., 2008) and alter essential marine habitats (Buchan, 2000; Gardner et al., 2003; Pandolfi et al., 2003; Jackson J. B. C et al., 2014), the high levels of genetic diversity observed were somewhat surprising. Indeed, genetic diversity measures for Hail Mary, an active but fished Nassau grouper FSA, were similar to those for the rest of The Bahamas, suggesting no loss in genetic diversity. In other exploited grouper species, e.g., *E. itajara*, molecular analyses employing microsatellites have provided evidence for reduced genetic variability (Silva-Oliveira et al., 2008). However, this is not always the case, as demonstrated by Zatcoff et al. (2004) for *E. morio* and Scamp, *Mycteroperca phenax*; in their study, both species were genetically homogeneous and genetically diverse, despite intense fishing pressure.

Typically, high heterozygosity and allelic richness is beneficial because, potentially, it confers a fitness advantage and has implications for both short and long-term adaptations to both natural and anthropogenic stressors (Bouzat, 2010; Bernatchez, 2016). We found no significant difference in allelic richness in fish sampled from across the islands, but these values were low and may indicate possible loses of genetic diversity (Pinsky and Palumbi, 2014). The allelic richness results reported here are analogous to other published data for Nassau grouper (Bernard et al., 2016) and *E. marginatus* (Schunter et al., 2011), but higher than values reported from *E. quernus* (Rivera et al., 2011). In populations that undergo a bottleneck, rare or unique alleles may be lost, deleterious alleles may become fixed and inbreeding is more likely to occur (Garza and Williamson, 2001). In our study, coefficients of inbreeding were high in Abaco ($F_{IS} = 0.098$) and Long Island ($F_{IS} = 0.117$), suggesting that inbreeding of Nassau grouper may be occurring at these locations. However, it is important to highlight that samples from both these islands showed possible deviations from Hardy-Weinberg Equilibrium. Additionally, sub-adults comprised the majority of Nassau grouper from Abaco and most of these fish were caught around the same area (**Table 1**). This is likely due to Wahlund effects (from the incorporation of mixed cohorts and/or population sub-structuring), providing some explanation for one of the highest F_{IS} values found in this study (**Table 2**, Supplementary Table 4). Similar patterns may not have been observed if Nassau grouper were sampled over a broader geographic area and across different life stages. In contrast, both sub-adults and adults were sampled over a wider area in Long Island, although mostly around the northern part of the island.

Effective Population Size and Population Bottlenecks

In most instances, LDNE derived N_e estimates varied in accordance with the critical allele frequency used, with the smallest allele frequency (0.005) and smaller sample sizes generating the lowest estimates for N_e (**Table 4**, Supplementary Table 7). Analysing the data by sampling year, life stage, and population greatly reduced sample sizes and produced mostly negative results, which is either an artifact of sampling or suggest that N_e is indistinguishable from infinity (Waples and Do, 2010; MacBeth et al., 2013). Recently, MacBeth et al. (2013) compared empirical and simulated data of various population sizes to estimate N_e and examine the accuracy of these estimates for Spanish mackerel (*Scomberomorus commerson*), demonstrating how sample size and the presence of conspecific migrants from

genetically differentiated populations could result in reduced or negative N_e estimates.

In the present study, contemporary values of N_e were generally low, but higher estimates were observed in Andros, Hail Mary, and Great Inagua (**Table 4**). M-ratio and *VarEff* analyses provided strong evidence in support of both recent and historic population declines. Recent bottlenecks were detected for Abaco, Eleuthera, Exuma and Hail Mary fish despite showing high levels of genetic diversity consistent with the rest of The Bahamas. This is particularly interesting for Hail Mary, which was also the location with the second highest N_e estimate (**Table 4**). Fishing pressure on Nassau grouper around Eleuthera and Exuma, and FSA fishing at Hail Mary are likely to have contributed to the observed bottlenecks. The majority of contemporary LDNE-based N_e values we derived are smaller than those reported (using different microsatellite markers) for *E. marginatus*—an endangered grouper species with similar life history characteristics to Nassau grouper (Schunter et al., 2011). However, the smallest contemporary N_e value observed (Abaco: 375) was similar to those reported for other teleosts (e.g., Santer and Slinger sea bream, *Cheimerius nufar* and *Chrysoblephus puniceus*; Coscia et al., 2016).

We recognize that most Nassau grouper used in this study were sampled over consecutive years and across life stages with a few exceptions (**Table 1**), thus potentially violating assumptions regarding discrete generations (Waples, 2006; Waples et al., 2014). Variability in N_e estimates has been linked to differences in reproductive success and/or the methods used in their computation (Hare et al., 2011; Waples et al., 2013, 2014; Coscia et al., 2016; Waples and Anderson, 2017). For example, Waples et al. (2014) demonstrated that N_e estimates based on a variety of age-structured samples are likely to be underestimated due to Wahlund effects. In the present study, both LDNE and *VarEff* produced similar estimates of contemporary N_e, providing additional confidence in modeled results. However, based on potential biases associated with N_e estimates, results should be interpreted with caution.

The timing of contemporary N_e losses (i.e., within the last 100 generations) coincides with known anthropogenic impacts, e.g., fishing, habitat degradation to Bahamian marine resources (Smith, 1972; Lang et al., 1988; Buchan, 2000; Jackson J. B. C et al., 2014). Historical estimates of Nassau grouper N_e were very large. Jue (2006) also reported a large historical N_e of ∼30,000 for Gag (*Mycteroperca microlepi*), another exploited aggregating grouper species. M-ratio values for Bahamian Nassau grouper were below the critical M (0.68) recommended by Garza and Williamson (2001), highlighting the severity of population declines. In contrast, Bernard et al. (2016) reported a weakly bottlenecked Nassau grouper population from an FSA in the USVI.

More remarkably, if we examine historical N_e reductions in The Bahamas, assuming a generation time of 9 years, the first signs of population decline would have occurred 3,600 year ago. Although Florida is within close proximity to The Bahamas, during the 1800s most of the population resided in northern Florida (Smith, 2005). Consequently, it seems unlikely that the native Indian tribes residing there 3,600 years ago would have traveled to The Bahamas to fish with access to ample marine resources around the shallow Floridian coast. The earliest known inhabitants to populate the Bahamian archipelago were the Lucayan or Arawak Indians (900–1500 AD) who survived by engaging in low technology subsistence fishing and farming, before their eradication in the 1500s (Buchan, 2000). A decline in N_e 3,600 years ago pre-dates the existence of human occupancy in The Bahamas and implies that historical population bottlenecks were likely due to natural as opposed to anthropogenic events.

According to marine geological records, four major glacial events happened during the Pleistocene (2.6 Ma to 11,700 years. ago), resulting in massive (120 m) fluctuations in sea level, which impacted The Bahamas (Sealey, 1994). This was punctuated by smaller variations in sea level (5–6 m) above current conditions during inter-glacial periods (White et al., 1998) that also would have affected marine organisms. Unfortunately, no historical genetic material was available to establish baseline genetic variability and cross-validate modeled estimates of temporal changes in the effective population sizes and contemporary genetic diversity values of Nassau grouper. However, previous studies have shown that the demography, distribution and genetic composition of species can be strongly influenced by geological processes and associated eustatic sea level changes (e.g., Hewitt, 2000; Pellissier et al., 2014; Brüniche-Olsen et al., 2017). For example, an analysis of >6,000 fish species showed strong correlations between current species richness and distribution patterns with historic climatic events that negatively impacted reef habitats (Pellissier et al., 2014). This finding was particularly pronounced for Caribbean reefs and strongly associated reef species, which experienced drastic reductions in water temperatures during past glacial events (White et al., 1998; Hewitt, 2000; Pellissier et al., 2014). Although speculative, it is possible that historical bottlenecks experienced by Bahamian Nassau grouper ∼3,600 years ago could have been associated with severe climatic perturbations. Collectively, these results suggest that both climate-driven biological processes and human exploitation have impacted the genetic composition of Nassau grouper.

Management Recommendations

The concept of management units (MUs) is based upon populations with genetically distinct population dynamics and demographics, resulting in management strategies tailored to preserve genetic variability within units (Palsbøll et al., 2007). Two putative genetic clusters were revealed by STRUCTURE analysis, but these do not appear related to geography. Possible explanations for this finding include panmixia, temporal differences in spawning between two sympatric stocks, and secondary contact from previously isolated Nassau grouper populations (e.g., Reid et al., 2016). However, our study was not designed to investigate these hypotheses, and to address it would require expanding spatial and temporal sampling of Nassau grouper throughout The Bahamas and the Caribbean. In the absence of demographic genetic structure, N_e is a viable alternative to use for conservation management. Prior to this study, no estimates of N_e were available for Nassau grouper.

Genetic analyses have been successfully used to demonstrate the genetic consequences of exploitation through reductions in N_e (e.g., Hauser et al., 2002; Hoarau et al., 2005; Poulsen et al., 2006), a finding we observed in the present study. The protection of FSAs has been identified as a biodiversity target for The Bahamas (Moultrie, 2012) and has been incorporated into Marxan analyses for the establishment of new MPAs (Moultrie, 2012; Moss and Moultrie, 2014). Given the substantial reduction in N_e and the low contemporary values of allelic richness, strengthened national, regional and international relationships are recommended to tackle illegal fishing, to eliminate FSA targeted fishing and to establish MPA networks to protect marine habitats that are of critical importance to rebuild Nassau grouper stocks.

CONCLUSIONS

Given that two putative genetic stocks have been identified for The Bahamas, over-harvesting of Nassau grouper may lead to reductions and losses in genetic diversity of the least abundant stock. The apparent lack of clear geographical or spatial population structure implies the need to adopt a conservative approach for managing Nassau grouper in The Bahamas until such time as the main drivers shaping the genetic composition of these fish are fully understood. Although estimates of genetic diversity were mostly high and fairly uniform, temporal analyses of changes in N_e show that contemporary populations have dramatically reduced effective population sizes compared to historic levels. Because N_e accuracy is affected by sample size, marker numbers, and marker polymorphism (Tallmon et al., 2010; Waples et al., 2014; Wang et al., 2016), performing cohort analysis, increasing the numbers of polymorphic molecular markers, sample size, and spatial coverage throughout the archipelago would allow for enhanced assessments of the genetic population dynamics of Nassau grouper. The application of more sophisticated molecular techniques than microsatellites, for example, RAD-sequencing, which can identify thousands of single nucleotide polymorphisms (SNPs), could help to resolve potential fine-scale intraspecific genetic structure (e.g., Larson et al., 2013). SNP data coupled with oceanographic and biophysical models and *in situ* biological data (Miller, 2007; Kough et al., 2016), would likely provide additional insight into the mechanisms influencing the spatial distribution, source-sink dynamics and connectivity of the species. The data presented provide an important foundation upon which future changes to N_e and genetic diversity can be monitored and compared to report on the status of management practices. Overall, this research represents the most comprehensive assessment of the genetic architecture of Nassau grouper in The Bahamas to date and has yielded novel insights into the historical processes that may have shaped contemporary genetic patterns. These findings highlight the utility of molecular approaches for enhancing management of endangered species.

AUTHOR CONTRIBUTIONS

KS designed and conducted fieldwork, coordinated sample collection, performed genomic DNA extractions and quality testing, primer optimisation, genotyping, microsatellite data analysis and wrote the manuscript. RK provided laboratory support, assisted with data analysis and contributed to writing the manuscript. CD conducted fieldwork and commented on the manuscript. SS, JS, and CT helped in the project design and to refine the manuscript. All authors reviewed and approved of the final version. We have no competing interests to declare. Feedback from two reviewers enhanced the manuscript.

FUNDING

Molecular research was financially supported by the University of Exeter and research cruises were funded by the John G. Shedd Aquarium. Partial funding for KS was provided by the Shirley Oakes Butler Charitable Trust, Rotary Club of East Nassau and a private donation by I. de la Rocha.

ACKNOWLEDGMENTS

Animal handing followed established and approved ethical procedures reviewed by Shedd Aquarium's internal and external research committee. All fin clip samples were obtained with permission from The Bahamas Department of Marine Resources under scientific research permit (MAMR/FIS/17–Sherman) to KS and follow UK Home Office guidelines. **Figure 1** was produced by Lindy Knowles. Special thanks to Kristine Stump, Randolph Burrows, Stephanie Buhler, Jocelyn Curtis-Quick, Candice Brittain, Tim Higgs, Jonisha Cartwright, Shenica Campbell, Andrieka Burrows, Felice Knowles, Catherine Booker, Annabelle Brooks, and Elizabeth Wallace for assisting with sample collection and to Keith Pamper and the crew of the R/V Coral Reef II for logistical support in preparation for and during research cruises. L. Knowles and A. Lundy (Bahamas National Trust), supported logistics for sample transportation and fieldwork.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fmars.2017.00393/full#supplementary-material

REFERENCES

Adams, C. E., Bean, C. W., Dodd, J. A., Down, A., Etheridge, E. C., Gowans, A. R. D., et al. (2016). Inter- and intra-population phenotypic and genotypic structuring in the European whitefish *Coregonus lavaretus*, a rare freshwater fish in Scotland. *J. Fish Biol.* 88, 580–594. doi: 10.1111/jfb.12855

Aguilar-Perera, A. (2006). Disappearance of a Nassau grouper spawning aggregation off the southern Mexican Caribbean coast. *Mar. Ecol. Prog. Ser.* 327, 289–296. doi: 10.3354/meps327289

Albins, M. A., Hixon, M. A., and Sadovy, Y. (2009). Threatened fishes of the world: *Epinephelus striatus* (Bloch, 1792) (Serranidae). *Environ. Biol. Fishes* 86, 309–310. doi: 10.1007/s10641-009-9512-5

Allendorf, F. W., England, P. R., Luikart, G., Ritchie, P. A., and Ryman, N. (2008). Genetic effects of harvest on wild animal populations. *Trends Ecol. Evol.* 23, 327–337. doi: 10.1016/j.tree.2008.02.008

Archer, S. K., Allgeier, J. E., Semmens, B. X., Heppell, S. A., Pattengill-Semmens, C. V., Rosemond, A. D., et al. (2015). Hot moments in spawning aggregations: implications for ecosystem-scale nutrient cycling. *Coral Reefs* 34, 19–23. doi: 10.1007/s00338-014-1208-4

Beldade, R., Jackson, A. M., Cudney-Bueno, R., Raimondi, P. T., and Bernardi, G. (2014). Genetic structure among spawning aggregations of the gulf coney *Hyporthodus acanthistius*. *Mar. Ecol. Prog. Ser.* 499, 193–201. doi: 10.3354/meps10637

Bernard, A. M., Feldheim, K. A., Nemeth, R., Kadison, E., Blondeua, J., Semmens, B. X., et al. (2016). The ups and downs of coral reef fishes: the genetic characteristics of a formerly severely overfished but currently recovering Nassau grouper fish spawning aggregation. *Coral Reefs* 35, 273–284. doi: 10.1007/s00338-015-1370-3

Bernard, A. M., Feldheim, K. A., Richards, V. P., Nemeth, R. S., and Shivji, M. S. (2012). Development and characterization of fifteen novel microsatellite loci for the Nassau grouper (*Epinephelus striatus*) and their utility for cross-amplification on a suite of closely related species. *Consererv. Genet. Res.* 4, 983–986. doi: 10.1007/s12686-012-9688-4

Bernatchez, L. (2016). On the maintenance of genetic variation and adaptation to environmental change: considerations from population genomics in fishes. *J. Fish Biol.* 89, 2519–2556. doi: 10.1111/jfb.13145

Bolden, S. K. (2000). Long-distance movement of a Nassau grouper (*Epinephelus striatus*) to a spawning aggregation in the central Bahamas. *Fish Bull.* 98, 642–645. Available online at: https://www.st.nmfs.noaa.gov/spo/FishBull/983/12.pdf

Bouzat, J. L. (2010). Conservation genetics of population bottlenecks: the role of chance, selection, and history. *Conserv. Genet.* 11, 463–478. doi: 10.1007/s10592-010-0049-0

Brüniche-Olsen, A., Hazlitt, S. L., and Eldridge, M. (2017). Genetic evidence of range-wide population declines in an Australian marsupial prior to European settlement. *Conserv. Genet.* 18, 1–13. doi: 10.1007/s10592-017-0960-8

Buchan, K. C. (2000). The Bahamas. *Mar. Poll. Bull.* 41, 94–111. doi: 10.1016/S0025-326X(00)00104-1

Buchholz-Sørensen, M., and Vella, A. (2016). Population structure, genetic diversity, effective population size, demographic history and regional connectivity patterns of the endangered dusky grouper, *Epinephelus marginatus* (Teleostei: Serranidae), within Malta's fisheries management zone. *PLoS ONE* 11:e0159864. doi: 10.1371/journal.pone.0159864

Carvalho, G. R., and Hauser, L. (1994). Molecular-Genetics and the stock concept in fisheries. *Rev. Fish Biol. Fish.* 4, 326–350.

Carvalho, G. R., and Hauser, L. (1998). Advances in the molecular analysis of fish population structure. *Ital. J. Zool.* 65, 21–33. doi: 10.1080/11250009809386791

Cheung, W. W. L., Sadovy, Y., Braynen, M. T., and Gittens, L. G. (2013). Are the last remaining Nassau grouper *Epinephelus striatus* fisheries sustainable? Status quo in the Bahamas. *Endanger. Species Res.* 20, 27–39. doi: 10.3354/esr00472

Choat, J. H. (2012). "Spawning aggregations in reef fishes: ecological and evolutionary processes," in Reef *Fish Spawning Aggregations*: *Biology, Research* and *Management*, eds Y. Sadovy de Mitcheson and P. L. Colin (New York, NY: Springer), 85–116.

Coleman, F. C., Koenig, C. C., Scanlon, K. M., Heppell, S., Heppell, S., and Miller, M. W. (2010). Benthic Habitat Modification through Excavation by Red Grouper, *Epinephelus morio*, in the Northeastern Gulf of Mexico. *Open Fish Sci. J.* 3, 1–15. doi: 10.2174/1874401X01003010001

Colin, P. L. (1992). Reproduction of the Nassau grouper, *Epinephelus striatus*, (Pisces: Serranidae) and its relationship to environmental conditions. *Environ. Biol. Fish.* 34, 357–377. doi: 10.1007/BF00004740

Colin, P. L. (1995). Surface currents in Exuma Sound, Bahamas and adjacent areas with reference to potential larval transport. *Bull. Mar. Sci.* 56, 48–57.

Colin, P. L. (2012). "Aggregation spawning: biological aspects of the early life history," in Reef *Fish Spawning Aggregations*: *Biology, Research* and *Management*, eds Y. Sadovy de Mitcheson and P. L. Colin (New York, NY: Springer), 191–224.

Colin, P. L., Laroche, W. A., and Brothers, E. B. (1997). Ingress and settlement in the Nassau grouper, *Epinephelus striatus* (Pisces: Serranidae), with relationship to spawning occurrence. *Bull. Mar. Sci.* 60, 656–667

Cornish, A., and Eklund, A.-M. (2003). *Epinephelus Striatus*. The IUCN Red List of Threatened Species. e.T7862A12858266. Available online at: www.iucnredlist.org.

Coscia, I., Chopelet, J., Waples, R. S., Mann, B. Q., and Mariani, S. (2016). Sex change and effective population size: implications for population genetic studies in marine fish. *Heredity* 117, 251–258. doi: 10.1038/hdy.2016.50

Dahlgren, C. P., Buch, K., Rechisky, E., and Hixon, M. A. (2016a). Multiyear tracking of Nassau grouper spawning migrations. *Mar. Coast. Fish.* 8, 522–535. doi: 10.1080/19425120.2016.1223233

Dahlgren, C. P., Sherman, K., Lang, J., Kramer, P. R., and Marks, K. (2016b). *Bahamas Coral Reef Report Card*, *Vol. 1: 2011–2013*. Nassau. Available online at: http://www.agrra.org/wp-content/uploads/2016/05/Bahamas-2016-Coral-Reef-Report-Card.pdf

Davey, J. W., Hohenlohe, P. A., Etter, P. D., Boone, J. Q., Catchen, J. M., and Blaxter, M. L. (2011). Genome-wide genetic marker discovery and genotyping using next-generation sequencing. *Nat. Rev. Genet.* 12, 499–510. doi: 10.1038/nrg3012

Dieringer, D., and Schlötterer, C. (2003). Microsatellite analyser (MSA): a platform independent analysis tool for large microsatellite data sets. *Mol. Ecol. Notes* 3, 167–169. doi: 10.1046/j.1471-8286.2003.00351.x

Domeier, M. L. (2012). "Revisiting spawning aggregations: definitions and challenges," in *Reef Fish Spawning Aggregations: Biology, Research and Management*, Vol. 35, eds Y. Sadovy de Mitcheson and P. L. Colin (Dordrecht; Heidelberg; London; New York, NY: Springer), 1–20.

Earl, D. A., and vonHoldt, B. M. (2012). STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conserv. Genet. Res.* 4, 359–361. doi: 10.1007/s12686-011-9548-7

Estoup, A., Jarne, P., and Cornuet, J.-M. (2002). Homoplasy and mutation model at microsatellite loci and their consequences for population genetics analysis. *Mol. Ecol.* 11, 1591–1604. doi: 10.1046/j.1365-294X.2002.01576.x

Evanno, G., Regnaut, S., and Goudet, J. (2005). Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. *Mol. Ecol.* 14, 2611–2620. doi: 10.1111/j.1365-294X.2005.02553.x

Froese, R., and Pauly, D. (eds.) (2014). *FishBase*. Available online at: www.fishbase.org (Accessed Oct 23, 2014).

Garcia-Mayoral, E., Olsen, M., Hedeholm, R., Post, S., Nielsen, E. E., and Bekkevold, D. (2016). Genetic structure of West Greenland populations of lumpfish *Cyclopterus lumpus*. *J. Fish Biol.* 89, 2625–2642. doi: 10.1111/jfb.13167

Gardner, T. A., Côté, I. M., Gill, J. A., Grant, A., and Watkinson, A. R. (2003). Long-term region-wide declines in Caribbean corals. *Science* 301, 958–960. doi: 10.1126/science.1086050

Garza, J. C., and Williamson, E. G. (2001). Detection of reduction in population size using data from microsatellite loci. *Mol. Ecol.* 10, 305–318. doi: 10.1046/j.1365-294x.2001.01190.x

Gunn, J. T., and Watt, D. R. (1982). On the currents and water masses north of the Antilles/Bahamas Arc. *J. Mar. Res.* 40, 1–48

Hamilton, P. B., and Tyler, C. R. (2008). PERMANENT GENETIC RESOURCES: identification of microsatellite loci for parentage analysis in roach *Rutilus rutilus* and eight other cyprinid fish by cross-species amplification, and a novel test for detecting hybrids between roach and other cyprinids. *Mol. Ecol. Res.* 8, 462–465. doi: 10.1111/j.1471-8286.2007.01994.x

Hamner, W. M., and Largier, J. L. (2012). "Oceanography of the planktonic stages of aggregation spawning reef fishes," in Reef *Fish Spawning Aggregations*: *Biology, Research* and *Management*, eds Y. Sadovy de Mitcheson and P. L. Colin (New York, NY: Springer), 159–190.

Hare, M. P., Nunney, L., Schwartz, M. K., Ruzzante, D. E., Burford, M., Waples, R. S., et al. (2011). Understanding and estimating effective population size for practical application in marine species management. *Conserv. Biol.* 25, 438–449. doi: 10.1111/j.1523-1739.2010.01637.x

Hateley, J. G. (1995). Preliminary results of a protein electrophoretic analysis of genetic variation, population structure and gene flow in the Nassau grouper, *Epinephelus striatus*. *Proc. Gulf Carib. Fish. Inst.* 47, 888–905.

Hauser, L., Adcock, G. J., Smith, P. J., Bernal Ramírez, B., and Carvalho, G. R. (2002). Loss of microsatellite diversity and low effective population size in an

overexploited population of New Zealand snapper (*Pagrus auratus*). *Proc. Nat. Acad. Sci. U.S.A.* 99, 11742–11747. doi: 10.1073/pnas.172242899

Heemstra, P. C., and Randall, J. E. (1993). *FAO Species Catalogue, Vol. 16. Groupers of the World (family Serranidae, Subfamily Epinephelinae): An Annotated and Illustrated Catalogue of the Grouper, Rockcod, Hind, Coral Grouper, and Lyretail Species Known to Date*. FAO Fisheries Synopsis No. 125, Rome: Food and Agriculture Organization of the United Nations.

Hellberg, M. E. (2009). Gene flow and isolation among populations of marine animals. *An. Rev. Ecol. Evol. Syst.* 40, 291–310. doi: 10.1146/annurev.ecolsys.110308.120223

Heppell, S. A., Semmens, B. X., Archer, S. K., Pattengill-Semmens, C. V., Bush, P. G., McCoy, C. M., et al. (2012). Documenting recovery of a spawning aggregation through size frequency analysis from underwater laser callipers measurements. *Biol. Conserv.* 155, 119–127. doi: 10.1016/j.biocon.2012.06.002

Heppell, S. A., Semmens, B. X., Pattengill-Semmens, C. V., Bush, P. G., Johnson, B. C., McCoy, C. M., et al. (2009). Tracking potential larval dispersal patterns from Nassau grouper aggregation sites: evidence for local retention and the 'importance of place'. *Proc. Gulf Carib. Fish. Inst.* 61, 325–327. Available online at: http://aquaticcommons.org/15740/1/GCFI_61-48.pdf

Heppell, S. A., Semmens, B. X., Pattengill-Semmens, C. V., Bush, P. G., Johnson, B. C., McCoy, C. M., et al. (2011). Oceanographic patterns associated with Nassau grouper aggregation spawn timing: shifts in surface currents on the nights of peak spawning. *Proc. Gulf Carib. Fish. Inst.* 63, 152–154. Available online at: http://aquaticcommons.org/15523/

Hewitt, G. (2000). The genetic legacy of the Quaternary ice ages. *Nature* 405: 907–913. doi: 10.1038/35016000

Hilborn, R., and Walters, C. J. (1992). *Quantitative Fisheries Stock Assessment*. Choice, Dynamics and Uncertainty. London: Chapman and Hall.

Hoarau, G., Boon, E., Jongma, D. N., Ferber, S., Palsson, J., Van der Veer, H. W., et al. (2005). Low effective population size and evidence for inbreeding in an overexploited flatfish, plaice (*Pleuronectes platessa* L). *Proc. R. Soc. Lon.* 272, 497–503. doi: 10.1098/rspb.2004.2963

Hubisz, M. J., Falush, D., Stephens, M., and Pritchard, J. K. (2009). Inferring weak population structure with the assistance of sample group information. *Mol. Ecol. Res.* 9, 1322–1332. doi: 10.1111/j.1755-0998.2009.02591.x

Jackson, A. M., Semmens, B. X., and Bernardi, G. (2012). Characterization and cross-species amplification of microsatellite markers in Nassau grouper (*Epinephelus striatus*). *Mol. Ecol. Resour.* 12, 972–974. Available online at: http://www.reef.org/db/publications/6337

Jackson, A. M., Semmens, B. X., Sadovy de Mitcheson, Y., Nemeth, R. S., Heppell, S. A., Bush, P. G., et al. (2014). Population structure and phylogeography in Nassau grouper (*Epinephelus striatus*), a mass-aggregating marine fish. *PLoS ONE* 9:e97508. doi: 10.1371/journal.pone.0097508

Jackson, J. B. C., Donovan, M. K., Cramer, K. L., and Lam, V. V. (2014). *Status and Trends of Caribbean Coral Reefs: 1970–2012*. Gland: Global Coral Reef Monitoring Network; IUCN.

Jombart, T., Devillard, S., and Balloux, F. (2010). Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. *BMC Genet.* 11:94. doi: 10.1186/1471-2156-11-94

Jones, A. G., Rosenqvist, G., Berglund, A., and Avise, J. C. (1999). Clustered microsatellite mutations in the pipefish *Syngnathus typhle*. *Genetics* 152, 1057–1063.

Jue, N. K. (2006). Exploring the structure of genetic variation and the influences of demography on effective population size in the gag grouper *Mycteroperca microlepi* (Goode and Bean). *J. Fish Biol.* 69, 217–224. doi: 10.1111/j.1095-8649.2006.01273.x

Kadison, E., Nemeth, R. S., Blondeau, J., Smith, T., and Calnan, J. (2010). Nassau grouper (*Epinephelus striatus*) in St. Thomas, US Virgin Islands, with evidence for a spawning aggregation site recovery. *Proc. Gulf Carib. Fish. Inst.* 62, 273–279. Available online at: http://nsgl.gso.uri.edu/flsgp/flsgpc09001/data/papers/039.pdf

Kalinowski, S. T. (2005). HP-RARE 1.0: A computer program for performing rarefaction on measures of allelic richness. *Mol. Ecol. Notes* 5:187. doi: 10.1111/j.1471-8286.2004.00845.x

Kough, A. S., Claro, R., Lindeman, K. C., and Paris, C. B. (2016). Decadal analysis of larval connectivity from Cuban Snapper (*Lutjanidae*) spawning aggregations based on biophysical modelling. *Mar. Ecol. Prog. Ser.* 550, 175–190. doi: 10.3354/meps11714

Lang, J. C., Wicklund, R. I., and Dill, R. F. (1988). Depth and habitat related bleaching of zooxanthellate reef organisms near Lee Stocking Island, Exuma Cays, Bahamas. *Proc. Sixth Intl. Coral Reef Symp.* 3, 269–274.

Larson, W. A., Seeb, L. W., Everett, M. V., Waples, R. K., Templin, W. D., and Seeb, J. E. (2013). Genotyping by sequencing resolved shallow population structure to inform conservation of Chinook salmon (*Oncorhynchus tshawytscha*). *Evol. Appl.* 7, 355–369. doi: 10.1111/eva.12128

Luikart, G., Ryman, N., Tallmon, D. A., Schwartz, M. K., and Allendorf, F. W. (2010). Estimation of census and effective population sizes: the increasing usefulness of DNA-based approaches. *Conserv. Genet.* 11, 255–373. doi: 10.1007/s10592-010-0050-7

MacBeth, G. M., Broderick, D., Buckworth, R. C., and Ovenden, J. R. (2013). Linkage disequilibrium estimation of effective population size with immigrants from divergent populations: a case study on Spanish Mackerel (*Scomberomorus commerson*). *G3 Genes, Genomes, Genet.* 3, 709–717. doi: 10.1534/g3.112.005124

Maggio, T., Andaloro, F., and Arculeo, M. (2006). Genetic population structure of *Epinephelus marginatus* (Pisces, Serranidae) revealed by two molecular markers. *Ital. J. Zool.* 73, 275–283. doi: 10.1080/11250000600804599

Miller, T. J. (2007). Contribution of individual-based coupled physical–biological models to understanding recruitment in marine fish populations. *Mar. Ecol. Prog. Ser.* 347, 127–138. doi: 10.3354/meps06973

Moss, S., and Moultrie, S. (2014). *Ecological Gap Analysis: GEF FSP Sustainable Network of MPAs–The Bahamas*. Nassau: BEST Commission, 60.

Moultrie, S. (2012). *Master Plan for The Bahamas National Protected Area System*. Nassau: The Nature Conservancy; Northern Caribbean Office.

Nikolic, N., and Chevalet, C. (2014). Detecting past changes of effective population size. *Evol. Appl.* 7, 663–681. doi: 10.1111/eva.12170

O'Reilly, P. T., Canino, M. F., Bailey, K. M., and Bentzen, P. (2004). Inverse relationship between F_{st} and microsatellite polymorphism in the marine fish, walleye pollock (*Theragra chalcogramma*): implications for resolving weak population structure. *Mol. Ecol.* 13, 1799–1814. doi: 10.1111/j.1365-294X.2004.02214.x

Olsen, D. A., and LaPlace, J. A. (1979). A study of a Virgin Islands grouper fishery based on a breeding aggregation. *Proc. Gulf Carib. Fish. Inst.* 31, 130–144.

Ovenden, J. R., Leigh, G. M., Blower, D. C., Jones, A. T., Moore, A., Bustamante, C., et al. (2016). Can estimates of genetic effective population size contribute to fisheries stock assessments? *J. Fish Biol.* 89, 2505–2518. doi: 10.1111/jfb.13129

Paddack, M. J., Reynolds, J. D., Aguilar, C., Appelodoorn, R. S., Beets, J., Burkett, E. W., et al. (2009). Recent region-wide declines in Caribbean reef fish abundance. *Curr. Biol.* 19, 590–595. doi: 10.1016/j.cub.2009.02.041

Palsbøll, P. J., Bérubé, M., and Allendorf, F. W. (2007). Identification of management units using population genetic data. *Trends Ecol. Evol.* 22, 11–16. doi: 10.1016/j.tree.2006.09.003

Pandolfi, J. M., Bradbury, R. H., Sala, E., Hughes, T. P., Bjorndal, K. A., Cooke, R. G., et al. (2003). Global trajectories of the long-term decline of coral reef ecosystems. *Science* 301, 955–958. doi: 10.1126/science.1085706

Paris, C. B. (2009). "Fate of reef fish larvae trough ontogeny: advection or true mortality? theme session t: death in the sea," in *Proceedings of the 2009 Annual Science Conference, September 21–25 2009,* Berlin: ICES CM 2009/T: 13, 22.

Paris, C. B., Helgers, J., van Sebille, E., and Srinivasan, A. (2013). Connectivity modeling system: a probabilistic modeling tool for the multi-scale tracking of biotic and abiotic variability in the ocean. *Environ. Model. Softw.* 42, 47–54. doi: 10.1016/j.envsoft.2012.12.006

Peakall, R., and Smouse, P. E. (2006). GENALEX 6: genetic analysis in Excel. Population genetic software for teaching and research. *Mol. Ecol. Notes* 6, 288–295. doi: 10.1111/j.1471-8286.2005.01155.x

Peakall, R., and Smouse, P. E. (2012). GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research–an update. *Bioinformatics* 28, 2537–2539. doi: 10.1093/bioinformatics/bts460

Peery, M. Z., Kirby, R., Reid, B. N., Stoelting, R., Doucet-Bëer, E., Robinson, S., et al. (2012). Reliability of genetic bottleneck tests for detecting recent population declines. *Mol. Ecol.* 21, 3403–3418. doi: 10.1111/j.1365-294X.2012.05635.x

Pellissier, L., Leprieur, F., Parravicini, V., Cowman, P. F., Kulbicki, M., Litsios, G., et al. (2014). Quaternary coral reef refugia preserved fish diversity. *Science* 344, 1016–1019. doi: 10.1126/science.1249853

Pinsky, M. L., and Palumbi, S. R. (2014). Meta-analysis reveal lower genetic diversity in overfished populations. *Mol. Ecol.* 23, 29–39. doi: 10.1111/mec.12509

Poulsen, N. A., Nielsen, E. E., Schieruo, M. H., Loeschcke, V., and Grønkjaer, P. (2006). Long-term stability and effective population size in North Sea and Baltic Sea cod (*Gadus morhua*). *Mol. Ecol.* 15, 321–331. doi: 10.1111/j.1365-294X.2005.02777.x

Pritchard, J. K., Stephens, M., and Donnelly, P. (2000). Inference of population structure using multilocus genotype data. *Genetics* 155, 945–959.

Reid, K., Hoareau, T. B., Graves, J. E., Potts, W. M., Dos Santos, S. M., Klopper, A. W., et al. (2016). Secondary contact and asymmetrical gene flow in a cosmopolitan marine fish across the Benguela upwelling zone. *Heredity* 117, 307–315. doi: 10.1038/hdy.2016.51

Rhodes, K. L., Lewis, R. I., Chapman, R. W., and Sadovy, Y. (2003). Genetic structure of camouflage grouper, *Epinephelus polyphekadion* (Pisces: Serranidae), in the western central Pacific. *Mar. Biol.* 142, 771–776. doi: 10.1007/s00227-002-1002-7

Rivera, A. M. J., Kelley, C. D., and Roderick, G. K. (2004). Subtle population genetic structure in the Hawaiian grouper, *Epinephelus quernus* (Serranidae) as revealed by mitochondrial DNA analyses. *Biol. J. Linn. Soc.* 81, 449–468. doi: 10.1111/j.1095-8312.2003.00304.x

Rivera, M. A. J., Andrews, K. R., Kobayashi, D. R., Wren, J. L. K., Kelley, C., Roderick, G. K., et al. (2011). Genetic analyses and simulations of larval dispersal reveal distinct populations and directional connectivity across the range of the Hawaiian Grouper (*Epinephelus quernus*). *J. Mar. Biol.* 2011, 1–11. doi: 10.1155/2011/765353

Rousset, F. (2008). Genepop'007: a complete re-implementation of the genepop software for Windows and Linux. *Mol. Ecol. Res.* 8, 103–106. doi: 10.1111/j.1471-8286.2007.01931.x

Sadovy, Y., and Colin, P. L. (1995). Sexual development and sexuality in the Nassau grouper. *J. Fish Biol.* 46, 961–976. doi: 10.1111/j.1095-8649.1995.tb01401.x

Sadovy, Y., and Domeier, M. (2005). Are aggregation-fisheries sustainable? Reef fish fisheries as a case study. *Coral Reefs* 24, 254–262. doi: 10.1007/s00338-005-0474-6

Sadovy, Y., and Eklund, A. M. (1999). *Synopsis of Biological Data on the Nassau Grouper, Epinephelus striatus (Bloch, 1792) and the Jewfish, E. itajara, Lichtenstein, 1822*. Seattle, WA, NOAA; National Marine Fisheries Service, 1–64.

Sadovy de Mitcheson, Y., Craig, M. T., Bertoncini, A. A., Carpenter, K. E., Cheung, W. W. L., Choat, J. H., et al. (2013). Fishing groupers towards extinction: a global assessment of threats and extinction risks in a billion dollar fishery. *Fish Fish.* 14, 119–136. doi: 10.1111/j.1467-2979.2011.00455.x

Sadovy De Mitcheson, Y., Cornish, A., Domeier, M., Colin, P. L., Russell, M., and Lindeman, K. C. (2008). A global baseline for spawning aggregations of reef fishes. *Conserv. Biol.* 22, 1233–1244. doi: 10.1111/j.1523-1739.2008.01020.x

Sala, E., Ballesteros, E., and Starr, R. M. (2001). Rapid decline of nassau grouper spawning aggregations in belize: fishery management and conservation needs. *Fisheries* 26, 23–30. doi: 10.1577/1548-8446(2001)026<0023:RDONGS>2.0.CO;2

Schunter, C., Carreras-Carbonell, J., Planes, S., Sala, E., Ballesteros, E., Zabala, M., et al. (2011). Genetic connectivity patterns in an endangered species: the dusky grouper (*Epinephelus marginatus*). *J. Exp. Mar. Biol. Ecol.* 401, 126–133. doi: 10.1016/j.jembe.2011.01.021

Sealey, N. E. (1994). *Bahamian Landscapes. An Introduction to the Geography of the Bahamas*, 2nd Edn., Nassau: Media Enterprises, 128.

Selkoe, K. A., D'Aloia, C. C., Crandall, E. D., Iacchei, M., Liggins, L., Puritz, J. B., et al. (2016). A decade of seascape genetics: contributions to basic and applied marine connectivity. *Mar. Ecol. Prog. Ser.* 554, 1–19. doi: 10.3354/meps11792

Shenker, J. M., Maddox, E. D., Wishinski, E., Pearl, A., Thorrold, S. R., and Smith, N. (1993). Onshore transport of settlement-stage Nassau grouper *Epinephelus striatus* and other fishes in Exuma Sound, Bahamas. *Mar. Ecol. Prog. Ser.* 98, 31–43. doi: 10.3354/meps098031

Sherman, K. D., Dahlgren, C. P., Stevens, J. R., and Tyler, C. R. (2016). Integrating population biology into conservation management for endangered Nassau grouper *Epinephelus striatus*. *Mar. Ecol. Prog. Ser.* 554, 263–280. doi: 10.3354/meps11771

Shimoda, N., Knapik, E. W., Ziniti, J., Sim, C., Yamada, E., Kaplan, S., et al. (1999). Zebrafish genetic map with 2000 microsatellite markers. *Genomics* 58, 219–232. doi: 10.1006/geno.1999.5824

Silva-Oliveira, G. C., do Rêgo, P. S., Schneider, H., Sampaio, I., and Vallinoto, M. (2008). Genetic characterisation of populations of the critically endangered Goliath grouper (*Epinephelus itajara*, Serranidae) from the Northern Brazilian coast through analyses of mtDNA. *Genet. Mol. Biol.* 31, 988–994. doi: 10.1590/S1415-47572008005000013

Smith, C. L. (1972). A spawning aggregation of Nassau grouper, *Epinephelus striatus* (Bloch). *Trans. Am. Fish. Soc.* 101, 257–261. doi: 10.1577/1548-8659(1972)101<257:ASAONG>2.0.CO;2

Smith, P. J., Francis, R. I. C. C., and McVeagh, M. (1991). Loss of genetic diversity due to fishing pressure. *Fish. Res.* 10, 309–316. doi: 10.1016/0165-7836(91)90082-Q

Smith, S. K. (2005). *Florida Population Growth: Past, Present and Future*. Bureau of Economic and Business Research, University of Florida, Gainesville, FL. Available online at: https://www.bebr.ufl.edu/sites/default/files/FloridaPop2005_0.pdf (Accessed Oct 13, 2017).

Starr, R. M., Sala, E., Ballesteros, E., and Zabala, M. (2007). Spatial dynamics of the Nassau grouper *Epinephelus striatus* in a Caribbean atoll. *Mar. Ecol. Prog. Ser.* 343, 239–249. doi: 10.3354/meps06897

Stevenson, D. E., Champman, R. W., and Sedberry, G. R. (1998). Stock identification in nassau grouper, *Epinephelus striatus*, using microsatellite DNA analysis. *Proc. Gulf Carib. Fish. Inst.* 50, 727–749

Storey, J. D., and Tibshirani, R. (2003). Statistical significance for genome-wide studies. *Proc. Natl. Acad. Sci. USA.* 100, 9440–9445. doi: 10.1073/pnas.1530509100

Stump, K., Dahlgren, C. P., Sherman, K. D., and Knapp, C. R. (2017). Nassau grouper migrations during full moon suggest collapsed historic fish spawning aggregation and evidence of undocumented aggregation. *Bull. Mar. Sci.* 93, 375–389. doi: 10.5343/bms.2016.1042

Tallmon, D. A., Gregovich, D., Waples, R. S., Scott Baker, C., Jackson, J., Taylor, B. L., et al. (2010). When are genetic methods useful for estimating contemporary abundance and detecting population trends? *Mol. Ecol. Res.* 10, 684–692. doi: 10.1111/j.1755-0998.2010.02831.x

Truett, G. E., Heeger, P., Mynatt, R. L., Truett, A. A., Walker, J. A., and Warman, M. L. (2000). Preparation of PCR-quality mouse genomic DNA with hot sodium hydroxide and tris. (HotSHOT). *Biotechniques* 29, 52–54.

Tucker, J. W. Jr., and Woodard, P. C. (1991). Growth and development of domestic juvenile Nassau groupers. *Proc. Gulf Carib. Fish. Inst.* 43, 389–391.

van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., and Shipley, P. (2004). MICRO-CHECKER: software for identifying and correcting genotyping errors in microsatellite data. *Mol. Ecol. Notes* 4:535. doi: 10.1111/j.1471-8286.2004.00684.x

Wallace, E. M. (2015). High intraspecific genetic connectivity in the Indo-Pacific bonefishes: implications for conservation and management. *Environ. Biol. Fishes* 98, 2173–2186. doi: 10.1007/s10641-015-0416-2

Wallace, E. M., and Tringali, M. D. (2016). Fishery composition and evidence of population structure and hybridization in the Atlantic bonefish species complex (*Albula* spp.). *Mar. Biol.* 163:142. doi: 10.1007/s00227-016-2915-x

Wang, J., Santiago, E., and Caballero, A. (2016). Prediction and estimation of effective population size. *Heredity* 117, 193–206. doi: 10.1038/hdy.2016.43

Waples, R. S. (2006). A bias correction for estimates of effective population size based on linkage disequilibrium at unlinked gene loci. *Conserv. Genet.* 7, 167–184. doi: 10.1007/s10592-005-9100-y

Waples, R. S., and Anderson, E. C. (2017). Purging putative siblings from population genetic data sets: a cautionary view. *Mol. Ecol.* 26, 1211–1224. doi: 10.1111/mec.14022

Waples, R. S., Antao, T., and Luikart, G. (2014). Effects of overlapping generations on linkage disequilibrium estimates of effective population size. *Genetics* 197, 769–780. doi: 10.1534/genetics.114.164822

Waples, R. S., and Do, C. (2008). LDNE: a program for estimating effective population size from data on linkage disequilibrium. *Mol. Ecol. Res.* 8, 753–756. doi: 10.1111/j.1755-0998.2007.02061.x

Waples, R. S., Do, C. (2010). Linkage disequilibrium estimates of contemporary Ne using highly variable genetic markers: a largely untapped resource for applied conservation and evolution. *Evol. Appl.* 3, 244–262. doi: 10.1111/j.1752-4571.2009.00104.x

Waples, R. S., Luikart, G., Faulkner, J. R., and Tallmon, D. A. (2013). Simple life-history traits explain key effective population size ratios across diverse taxa. *Proc. Royal Soc.* 280:20131339. doi: 10.1098/rspb.2013.1339

Waples, R. S., Punt, A. E., and Cope, J. M. (2008). Integrating genetic data into the management of marine resources: how we can do it better. *Fish Fish.* 9, 423–449. doi: 10.1111/j.1467-2979.2008.00303.x

Weir, B. S., and Cockerham, C. C. (1984). Estimating F-statistics for the analysis of population structure. *Evolution* 38: 1358–1370.

White, B., Curan, H. A., and Wilson, M. A. (1998). Bahamian coral reefs yield evidence of a brief sea-level lowstand during the last interglacial. *Carbon. Evap.* 13, 231–234.

Worm, B., Barbier, E. B., Beaumont, N., Duffy, J. E., Folke, C., Halpern, B. S., et al. (2006). Impacts of biodiversity loss on ecosystem services. *Science* 314, 787–790. doi: 10.1126/science.1132294

Wright, S. (1931). Evolution in Mendelian populations. *Genetics* 16, 97–159.

Yue, G. H., David, L., and Orban, L. (2007). Mutation rate and pattern of microsatellites in common carp (*Cyprinus carpio* L.). *Genetica* 129, 329–331. doi: 10.1007/s10709-006-0003-8

Zatcoff, M. S., Ball, A. O., and Sedberry, G. R. (2004). Population genetic analysis of red grouper, *Epinephelus morio*, and scamp, *Mycteroperca phenax*, from the southeastern U.S. Atlantic and Gulf of Mexico. *Mar. Biol.* 144, 769–777. doi: 10.1007/s00227-003-1236-z

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

The reviewer RH and handling Editor declared their shared affiliation.

Supplementary Material

Historical Processes and Contemporary Anthropogenic Activities Influence Genetic Population Dynamics of Nassau Grouper (*Epinephelus striatus*) within The Bahamas

Krista D. Sherman, R. Andrew King, Craig P. Dahlgren, Stephen D. Simpson, Jamie R. Stevens* and Charles R. Tyler*

*Correspondence:

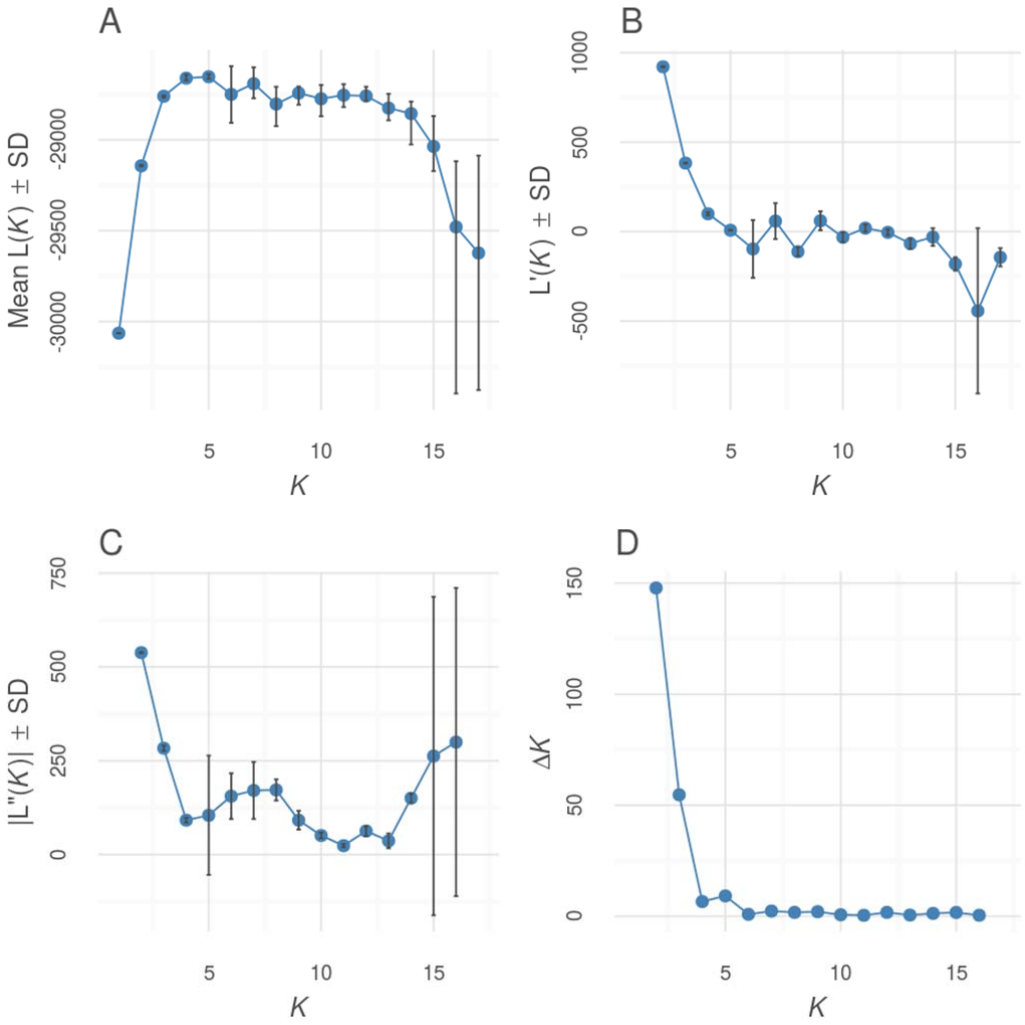
Dr. Jamie R. Stevens

J.R.Stevens@exeter.ac.uk

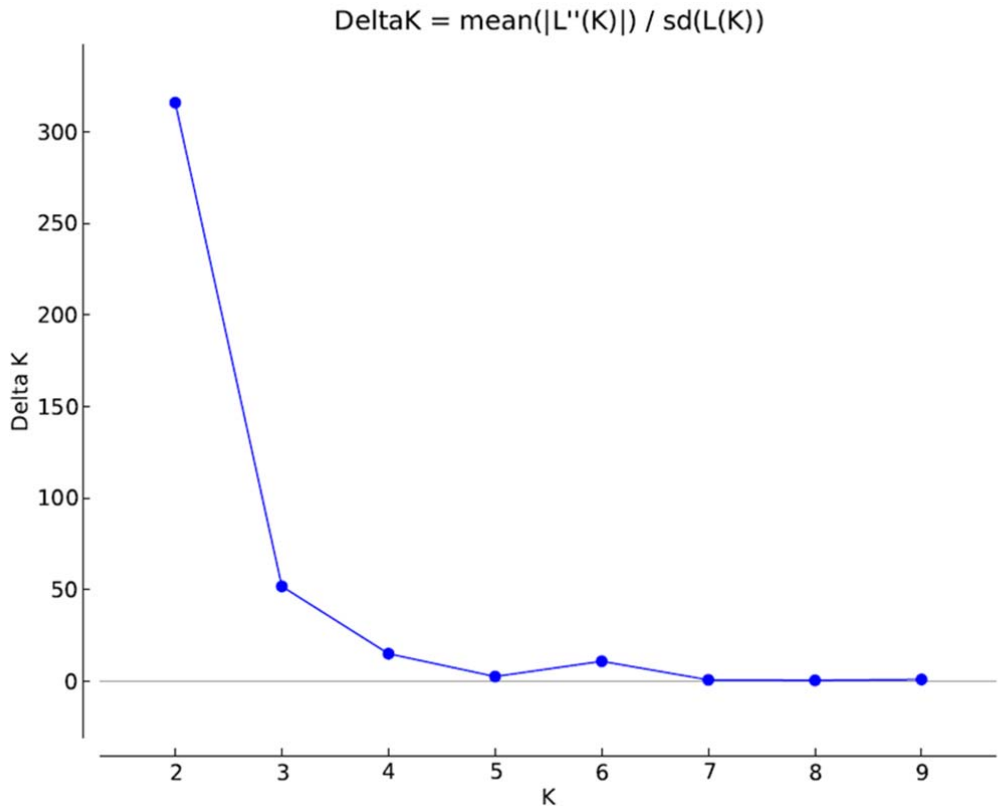
Professor Charles Tyler

C.R.Tyler@exeter.ac.uk

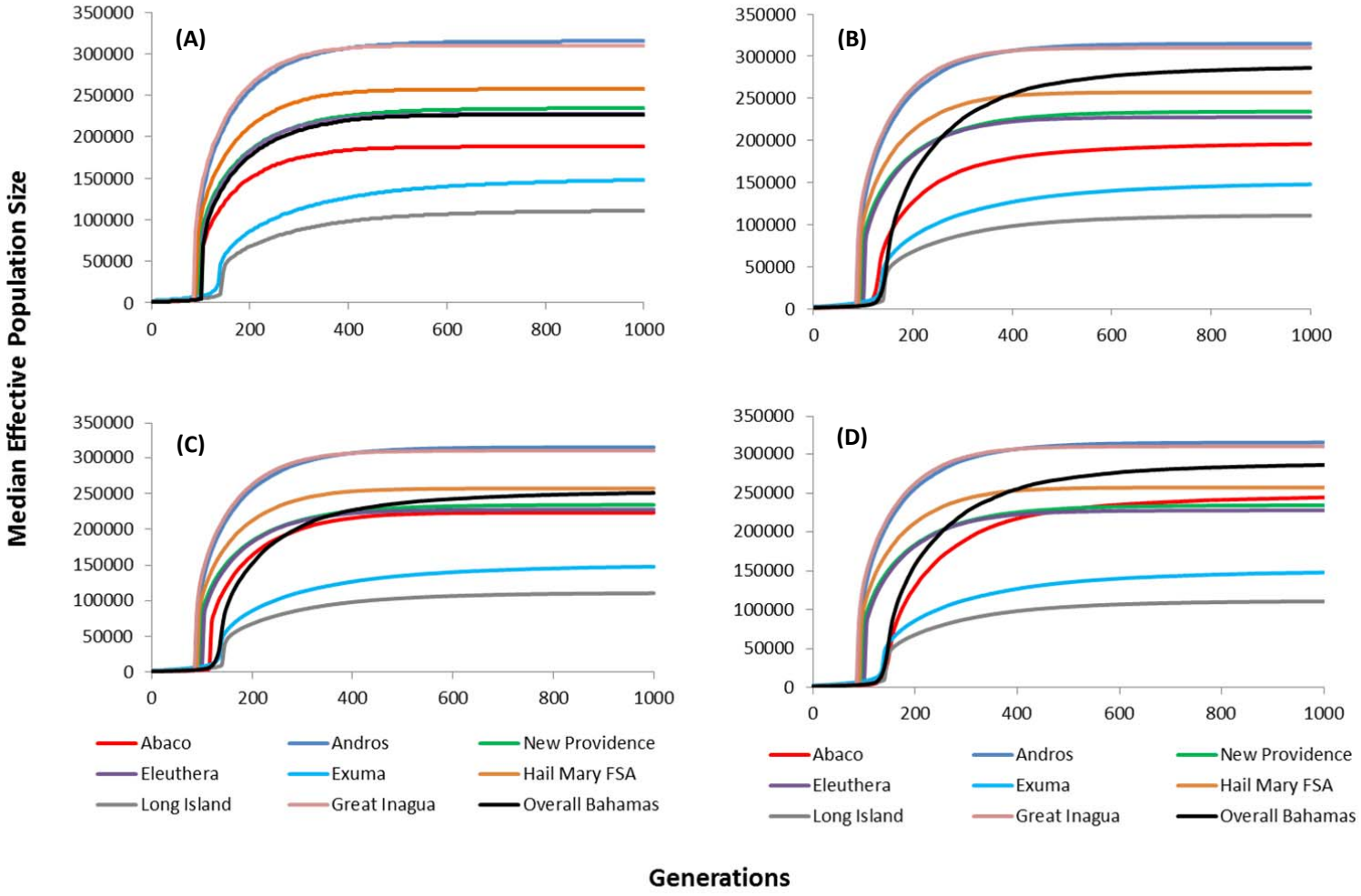
Supplementary Figures



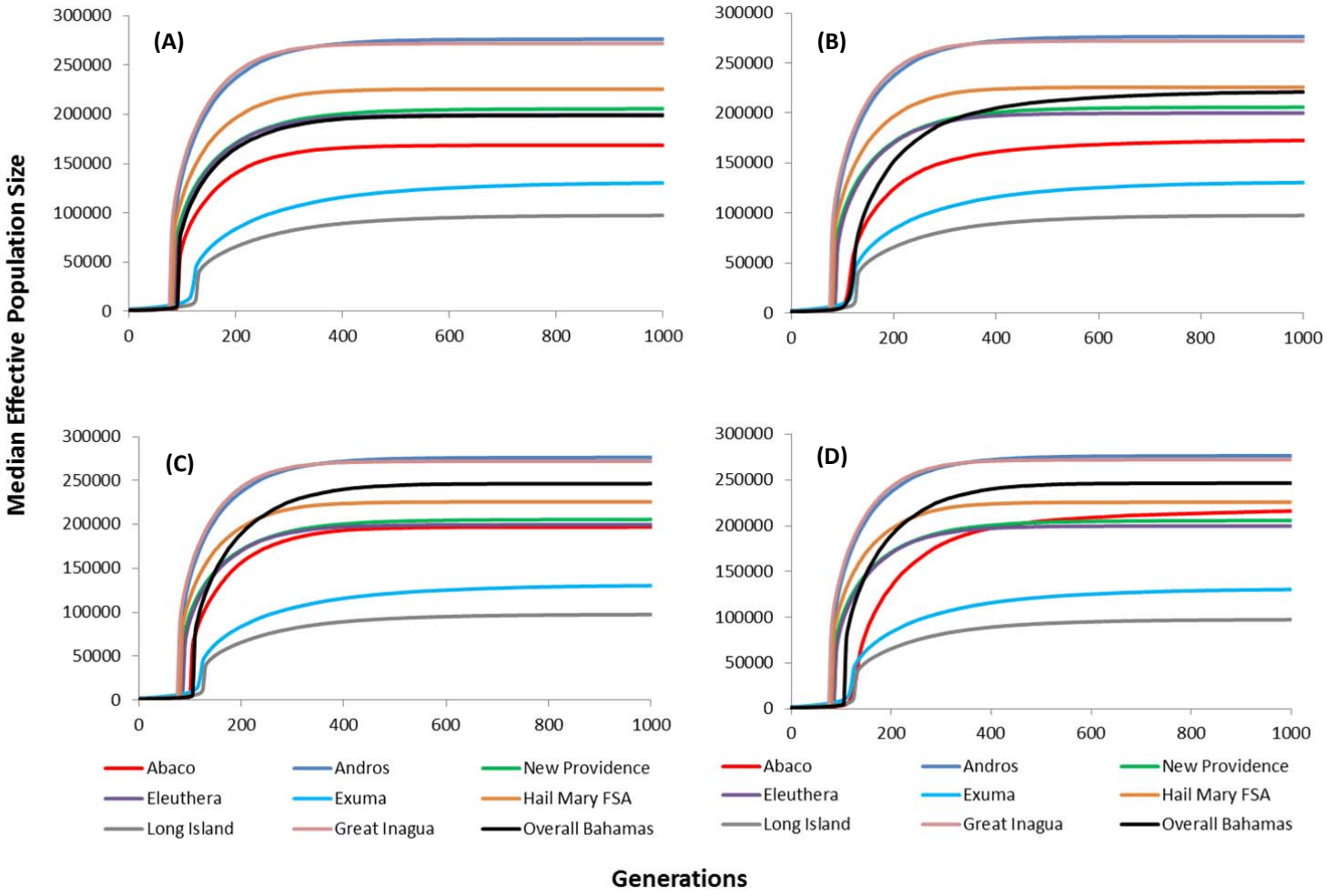
Supplementary Figure 1. Evanno results for *K* 1–17 calculated from ten independent runs in STRUCTURE under a pre- and post-burn-in of 75,000 and 150,000 Monte Carlo Markov Chain iterations depicting (a) mean ± standard deviation of log likelihoods, (b) rate of change of the likelihood distribution ± standard deviation, (c) absolute value of the second order rate of change of the likelihood distribution ± standard deviation, and (d) Evanno's statistic, showing $\Delta K = 2$.



Supplementary Figure 2. Evanno results for *K* 1–10 calculated from ten independent runs in STRUCTURE using a pre- and post-burn-in of 250,000 and 1,000,000 Monte Carlo Markov Chain iterations showing $\Delta K = 2$.

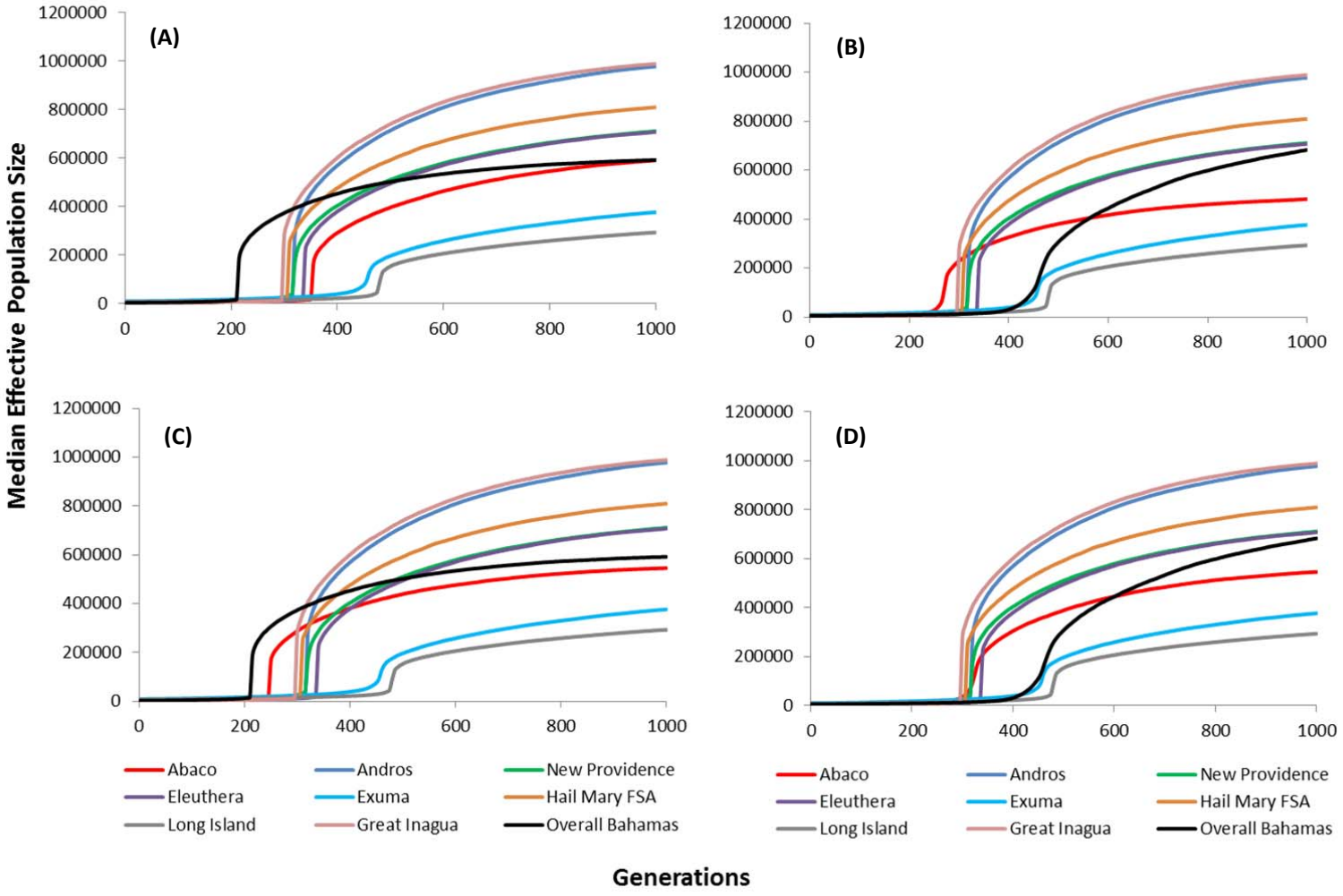


Supplementary Figure 3. *VarEff* estimates of temporal changes in the effective population sizes, N_e of Nassau grouper from Abaco (red), Andros (blue), New Providence (green), Eleuthera (purple), Exuma (blue), Hail Mary FSA (tan), Long Island (grey), Great Inagua (pink) and overall Bahamas (black) for the past 1,000 generations with a mutation rate, $\mu = 5 \times 10^{-4}$ where: (a) JMAX = 2 and (b) JMAX = 3 under NBAR = 10,000, and (c) JMAX = 2 and (d) JMAX = 3 under NBAR 15, 000.



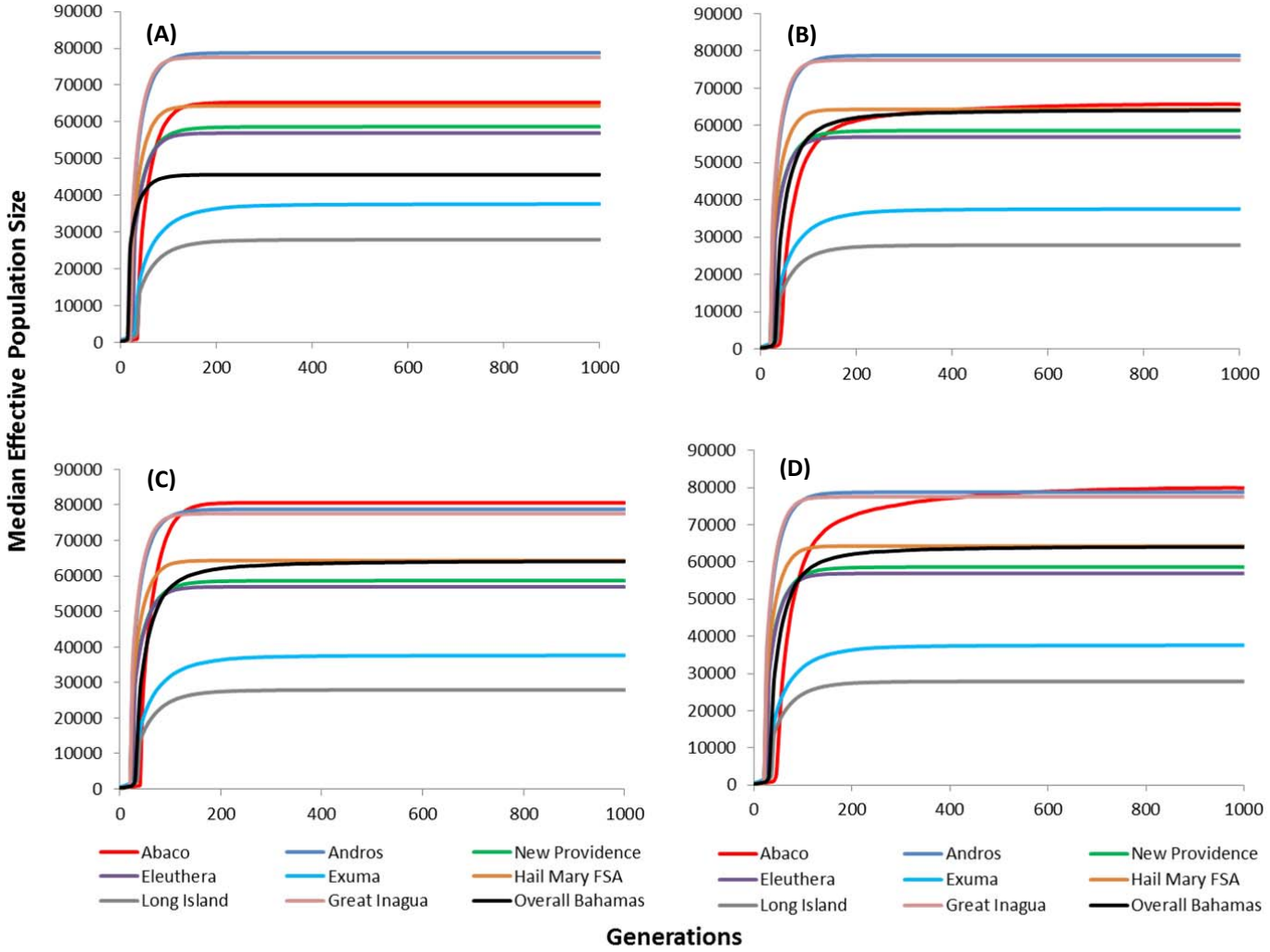
Supplementary Figure 4. *VarEff* estimates of temporal changes in the effective population sizes, N_e of Nassau grouper from Abaco (red), Andros (blue), New Providence (green), Eleuthera (purple), Exuma (blue), Hail Mary FSA (tan), Long Island (grey), Great Inagua (pink)

and overall Bahamas (black) for the past 1,000 generations with a mutation rate, $\mu = 5.57 \times 10^{-4}$ where: (a) JMAX = 2 and (b) JMAX = 3 under NBAR = 10,000, and (c) JMAX = 2 and (d) JMAX = 3 under NBAR 15, 000.



Supplementary Figure 5. *VarEff* estimates of temporal changes in the effective population sizes, N_e of Nassau grouper from Abaco (red), Andros (blue), New Providence (green), Eleuthera (purple), Exuma (blue), Hail Mary FSA (tan), Long Island (grey), Great Inagua (pink)

and overall Bahamas (black) for the past 1,000 generations with a mutation rate, $\mu = 1.5 \times 10^{-4}$ where: (a) JMAX = 2 and (b) JMAX = 3 under NBAR = 10,000, and (c) JMAX = 2 and (d) JMAX = 3 under NBAR 15, 000.



Supplementary Figure 6. *VarEff* estimates of temporal changes in the effective population sizes, N_e of Nassau grouper from Abaco (red), Andros (blue), New Providence (green), Eleuthera (purple), Exuma (blue), Hail Mary FSA (tan), Long Island (grey), Great Inagua (pink) and overall Bahamas (black) for the past 1,000 generations with a mutation rate, $\mu = 2.0 \times 10^{-3}$ where: (a) JMAX = 2 and (b) JMAX = 3 under NBAR = 10,000, and (c) JMAX = 2 and (d) JMAX = 3 under NBAR 15, 000.

Supplementary Table 1. Forward and reverse primer reaction volumes for the microsatellite loci used in each multiplex.

Multiplex	Locus	Primer Sequences (5'-3')	Dye	Forward	Reverse	Volumes
MP 1	EACD08	F: GAAAGGCAAAGCAGGTAAATA R: TTCAGGCAGTTTTATTGACAG	Blue (D4)	3	3	38 µl Primer
	Est33a	F: TGTGAGTCCTCCCTGTTTGA R: AGGTCACACAGCCACAGTGA	Green (D3)	5	5	64 µl H_2O
	Est416	F: AGCCTCAAACACTGCGGTA R: CCTGTCCAAGGTGCTGAAAC	Black (D2)	2	2	
	Est420	F:TGTGATAATGGTGGCATGTT R: TATGCCTTTCTGTGCGTGTG	Blue (D4)	3	3	
	Est338	F: TGATGAGGTGAAGTGTGGTTG R: CAATGCCAGGACCAAAGATT	Green (D3)	4	4	
	Est340	F: GTTTTGTCAGTGCCTCAGCA R: GAACACTTTTACTGCCCTCCA	Black (D2)	2	2	
MP 2	Est360	F:TTCACACAAGGTCACAAGAGAA R: GCAGTTTGTGTGCCCTCTTT	Blue (D4)	2	2	37.6 µl Primer
	Est92	F: GCAGATTGGAGCATGTGAAA R: CACAATTCCAGCAGAGAGCA	Green (D3)	3	3	62.4 µl H_2O
	EACB6	F: CATACGAATTGTGGTGCATTAC R: CGTCTGGAATACTTTGCTCAG	Black (D2)	3	3	
	Est262	F: AAGAGGATTGCAGACCAGGA R: CTCACCAATCTCACCCCAGT	Blue (D4)	0.8	0.8	
	EACD02	F:GTTGCAGAAATTGCAGAGAGAA R:TAAAGGCTGCTTCAGAGACATC	Green (D3)	5	5	
	Est290	F: CTGGCTCAGAGAGGCATTAAG R: ATGCTTGGTGAGTGCGTGT	Black (D2)	5	5	
MP 3	Est265	F: TGAAGTCATGTTCGCCTGAA R: GGAGGCTCTCTGTTCAGCAA	Blue (D4)	1	1	15.6 µl Primer
	Est376	F: GGCGTACCTGTCAAAAGAGG R: ATAATGTGGGCGTTCTGTGG	Green (D3)	0.8	0.8	84.4 µl H_2O
	Est267	F: GTGAGCGAGTTCATGGAGGA R: TCAACCAACCCCTTGAAAAG	Black (D2)	6	6	

Supplementary Table 2. Thermal cycling parameters for the PCR Protocol. The number of times a cycle was repeated is enclosed in square brackets.

Process	Temperature °C	Duration	Cycle
Denaturation	95	5:00	1 [1x]
Denaturation	95	0:30	2 [2x]
Annealing	62	0:30	
Extension	72	1:00	
Denaturation	95	0:30	3 [3x]
Annealing	58	0:30	
Extension	72	1:00	
Denaturation	95	0:30	4 [5x]
Annealing	55	0:30	
Extension	72	1:00	
Denaturation	95	0:30	5 [10x]
Annealing	53	0:30	
Extension	72	1:00	
Denaturation	95	0:30	6 [5x]
Annealing	51	0:30	
Extension	72	1:00	
Denaturation	95	0:30	7 [5x]
Annealing	49	0:30	
Extension	72	1:00	
Denaturation	95	0:30	8 [5x]
Annealing	47	0:30	
Extension	72	1:00	
Extension	72	10:00	9 [1x]
Extension	60	35:00	
Extension & Holding	10	60:00	

Supplementary Table 3. MICROCHECKER analysis of 454 Nassau grouper genotypes at 15 microsatellite loci.

Location	Est416	Est340	Est33a	Est338	EACD08	Est420	Est92	EACB6	Est360	EACD02	Est262	Est290	Est376	Est267	Est265
BI	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine
GB	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine
AB	Fine	***Null***	***Null***	***Null***	***Null***	Fine	Fine	***Null***	Fine	***Null***	Fine	***Null***	Fine	***Null***	***Null***
BIS	Fine	Fine	Fine	Fine	Fine	Fine	Fine	***Null***	Fine	***Null***	Fine	Fine	Fine	Fine	Fine
AN	Fine	Fine	Fine	Fine	Fine	Fine	Fine	***Null***	***Null***	Fine	Fine	Fine	Fine	Fine	Fine
NP	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine
EL	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	***Null***	Fine	***Null***	Fine
CI	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine	Fine
EX	Fine	Fine	Fine	Fine	***Null***	Fine	Fine	***Null***	***Null; Stuttering***	***Null***	Fine	Fine	Fine	Fine	Fine
HM	Fine	Fine	Fine	Fine	Fine	Fine	Fine	***Null***	Fine	Fine	Fine	Fine	Fine	Fine	Fine
LI	Fine	Fine	Fine	***Null***	Fine	Fine	***Null***	***Null***	***Null***	***Null***	***Null***	Fine	Fine	Fine	Fine
AK	Fine	Fine	Fine	Fine	Fine	Fine	Fine	***Null***	Fine	Fine	Fine	Fine	Fine	Fine	Fine
RI	Fine	Fine	Fine	Fine	Fine	Fine	Fine	***Null***	Fine	Fine	Fine	Fine	Fine	Fine	Fine
GI	Fine	Fine	Fine	Fine	Fine	Fine	Fine	***Null***	Fine	Fine	Fine	Fine	Fine	Fine	Fine
UK	Fine	Fine	Fine	Fine	***Null***	Fine	***Null***	***Null; Stuttering***	Null	Fine	***Null; Stuttering***	Fine	Fine	***Null***	***Null***
Total	**0**	**1**	**1**	**2**	**3**	**0**	**2**	**10**	**3**	**4**	**2**	**2**	**0**	**3**	**2**

Stutter Stuttering might have resulted in scoring errors

Null Homozygote excess suggests that null alleles may be present at this locus.

95% CI

Supplementary Table 4. Genetic diversity summary statistics for Nassau Grouper analysed by year, life stage, and population where, Na = number of effective alleles, A_R = allelic richness, PA_R = private allelic richness, H_O = observed heterozygosity, H_E = expected heterozygosity, HWE = probability of conforming to Hardy-Weinberg equilibrium, F_{IS} = inbreeding coefficient, N_g = number of genotypes. Global F_{ST} values are also provided with (significant values, $p \leq 0.05$ in bold). Note: A_R and PA_R for population two was computed with a minimum sample size of two genes per locus in HP-Rare v. 1.1.

Year	Location	Na	A_R	PA_R	H_O	H_E	HWE	F_{IS}	N_g	Global F_{ST}
2014										0.002082
	Andros	15.00	7.84	3.02	0.831	0.871	0.248	0.052	38	
	Hail Mary	11.00	7.48	2.66	0.813	0.852	0.432	0.058	19	
2015										-0.00037
	Andros	7.29	4.36	1.03	0.867	0.792	0.494	-0.050	7	
	Eleuthera	6.21	4.29	0.87	0.833	0.789	0.523	-0.011	6	
	Hail Mary	8.14	4.40	0.87	0.856	0.823	0.483	-0.013	10	
	Long Island	4.93	4.93	1.24	0.833	0.774	0.395	0.013	3	
2016										**0.003448**
	Abaco	17.64	6.19	0.65	0.782	0.865	0.020	0.098	75	
	Andros	5.36	5.36	0.28	0.886	0.746	0.597	-0.146	5	
	New Providence	8.43	6.18	0.46	0.872	0.825	0.362	-0.028	10	
	Eleuthera	11.71	5.97	0.36	0.820	0.843	0.120	0.037	31	
	Exuma	16.43	6.50	0.91	0.827	0.873	0.059	0.056	44	
	Hail Mary	11.50	5.97	0.37	0.856	0.844	0.406	-0.005	28	
	Long Island	11.79	6.52	1.07	0.763	0.861	0.337	0.124	18	
	Great Inagua	14.21	6.25	0.53	0.852	0.866	0.213	0.021	46	
	Unknown	13.93	6.10	0.41	0.796	0.859	0.019	0.080	55	
Life Stage										
Sub-adults	Eleuthera	10.93	6.92	2.40	0.813	0.838	0.145	0.041	29	**0.005373**
	Exuma	14.79	8.35	3.83	0.826	0.877	0.141	0.068	25	
Adults	Eleuthera	10.36	6.98	2.47	0.842	0.847	0.384	0.015	30	0.004404
	Exuma	11.00	7.42	2.91	0.828	0.846	0.245	0.033	19	
Population 1										-0.00054
	Abaco	11.57	6.31	0.21	0.792	0.843	0.383	0.064	52	
	Andros	11.07	6.59	0.34	0.857	0.842	0.543	-0.007	28	
	New Providence	7.21	6.48	0.36	0.862	0.804	0.380	-0.040	8	
	Eleuthera	11.29	6.38	0.18	0.829	0.849	0.321	0.027	58	
	Exuma	9.64	6.34	0.21	0.866	0.829	0.527	-0.035	20	
	Hail Mary	11.64	6.47	0.28	0.849	0.849	0.394	0.007	41	
	Long Island	7.29	6.52	0.4	0.815	0.809	0.445	0.024	8	
	Great Inagua	11.21	6.53	0.23	0.853	0.848	0.704	0.000	36	
Population 2										**0.011621**
	Abaco	10.42	1.92	0.96	0.803	0.866	0.266	0.104	10	
	Andros	11.36	1.91	0.88	0.864	0.870	0.329	0.028	12	

New Providence	–	–	–	–	–	–	–	0	–
Eleuthera	3.00	1.85	0.86	0.750	0.598	0.368	-0.164	2	
Exuma	13.07	1.89	0.85	0.803	0.868	0.165	0.089	19	
Hail Mary	6.14	1.88	0.81	0.864	0.791	0.501	-0.047	5	
Long Island	9.07	1.87	0.79	0.754	0.831	0.283	0.111	12	
Great Inagua	7.00	1.93	0.96	0.857	0.832	0.425	0.021	5	

Supplementary Table 5. Summary data of 14 microsatellite loci for Nassau grouper including allele size range, F-statistics and G-statistics per locus from Microsatellite Analyzer (MSA).

Locus	Allele Size Range (bp)	No. of Alleles	F_{ST}	F_{IT}	F_{IS}	G_{ST}	G_{ST}'
Est416	118-168	22	0.0023	-0.0029	-0.0052	0.0139	0.1091
Est340	184-276	34	0.0008	0.0186	0.0179	0.0212	0.2161
Est33a	109-157	21	0.0029	0.0617	0.0590	0.0123	0.0595
Est338	167-247	27	0.0006	0.0751	0.0745	0.0136	0.1368
EACD08	112-172	22	-0.0007	-0.0007	0.1374	0.0177	0.1013
Est420	181-243	24	0.0027	-0.0108	-0.0135	0.0074	0.0455
Est92	140-240	34	0.0023	0.0682	0.0661	0.0190	0.2069
Est360	130-240	33	0.0150	0.0812	0.0672	0.0189	0.1357
EACD02	243-341	42	0.0016	0.0699	0.0684	0.0156	0.2001
Est262	248-308	27	0.0049	0.0832	0.0786	0.0132	0.1159
Est290	320-378	26	0.0036	0.0827	0.0794	0.0208	0.1691
Est376	161-257	28	0.0016	0.0606	0.0591	0.0175	0.1417
Est267	179-279	26	-0.0005	0.0852	0.0857	0.0129	0.0982
Est265	145-245	28	-0.0037	0.0625	0.0659	0.0118	0.1110

Supplementary Table 6. Analysis of Molecular Variance (AMOVA) showing genetic variation in 421 sampled Nassau grouper throughout The Bahamas.

Summary AMOVA Results						
Source of Variation	**df**	**SS**	**MS**	**Est. Var.**	**%**	
Among Populations	8	68.138	8.517	0.019	0%	
Among Individuals	412	2773.185	6.731	0.586	10%	
Within Individuals	421	2340.500	5.559	5.559	90%	
Total	841	5181.823		6.165	100%	
F-Statistics	**Value**	**P(rand >= data)**				
F_{ST}	0.003	0.001				
F_{IS}	0.095	0.001				
F_{IT}	0.098	0.001				

Supplementary Table 7. LDNE effective population size, N_e estimates and 95% parametric confidence intervals for Nassau grouper for the pooled dataset, by sampling year, life stage and population for critical allele frequencies ranging from 0.05 to 0.005. N_g denotes the number of genotypes used for the analysis for each location.

Location	N_g	LDNE N_e (95% CI)	LDNE N_e (95% CI)	LDNE N_e (95% CI)	LDNE N_e (95% CI)
		0.05	*0.02*	*0.01*	*0.005*
Pooled Dataset					
Abaco	75	439.4(211.2 – ∞)	374.7(239.4 – 809.9)	318.3(229.5 – 506.7)	117.3(104.6 – 133.1)
Andros	50	885.4(243.5 – ∞)	2,978.2(481.1 – ∞)	148.6(120.5 – 191.5)	148.6(120.5 – 191.5)
New Providence	10	–888.3 (44.0 – ∞)	–888.3(44.0 – ∞)	–888.3(44.0 – ∞)	–888.3(44.0 – ∞)
Eleuthera	63	532.7(232.0 – ∞)	619.6(286.8 – ∞)	790.3(349.3 – ∞)	130.1(107.3 – 163.2)
Exuma	44	367.7(154.3 – ∞)	418.3(226.1 – 2216.1)	108.8(91.7 – 132.5)	108.8(91.7 – 132.5)
Hail Mary	57	–1,084.9(605.4 – ∞)	2,027.7(435.2 – ∞)	1,059.7 (398.7 – ∞)	940.0(416.7 – ∞)
Long Island	21	–704.0(160.4 – ∞)	779.4(158.1 – ∞)	779.4(158.1 – ∞)	779.4(158.1 – ∞)
Great Inagua	46	14,948.4(325.4 – ∞)	1,637.0(381.0 – ∞)	141.8(113.0 – ∞)	141.8(113.0 – ∞)
2014					
Andros	38	8,053.2(252.4 – ∞)	–938.4(649.5 – ∞)	183.3(131.9 – ∞)	183.3(131.9 – ∞)
Hail Mary	19	334.6(87.3 – ∞)	–1,431.2(176.2 – ∞)	–1,431.2(176.2 – ∞)	–1,431.2(176.2 – ∞)
2015					
Andros	7	–22.5(-72.8 – ∞)	–22.5(-72.8 – ∞)	–22.5(-72.8 – ∞)	–22.5(-72.8 – ∞)
Eleuthera	6	–32.8(52.5 – ∞)	–32.8(52.5 – ∞)	–32.8(52.5 – ∞)	–32.8(52.5 – ∞)
Hail Mary	10	–107.5(100.2 – ∞)	–107.5(100.2 – ∞)	–107.5(100.2 – ∞)	–107.5(100.2 – ∞)
Long Island	3	–4.7(-10.4 – ∞)	–4.7(-10.4 – ∞)	–4.7(-10.4 – ∞)	–4.7(-10.4 – ∞)
2016					
Abaco	75	439.4(211.2 – 100733.6)	374.7(239.4 – 809.9)	318.3(229.5 – 506.7)	117.3(104.6 – 133.1)
Andros	5	–27.9(22.1 – ∞)	–27.9(22.1 – ∞)	–27.9(22.1 – ∞)	–27.9(22.1 – ∞)
New Providence	10	–888.3(44.0 – ∞)	–888.3(44.0 – ∞)	–888.3(44.0 – ∞)	–888.3(44.0 – ∞)
Eleuthera	31	47.7(35.8 – 68.5)	56.0(42.9 – 78.6)	54.9(44.3 – 71.0)	54.9(44.3 – 71.0)
Exuma	44	367.7(154.3 – ∞)	418.3(226.1 – 2216.1)	108.8(91.7 – 132.5)	108.8(91.7 – 132.5)
Hail Mary	28	–288.7(385.5 – ∞)	–380.0(485.3 – ∞)	–669.5(411.8 – ∞)	–669.5(411.8 – ∞)
Long Island	18	–691.9(141.8 – ∞)	1,067.9(135.9 – ∞)	1,067.9(135.9 – ∞)	1,067.9(135.9 – ∞)
Great Inagua	46	14,948.4(325.4 – ∞)	1,637.0(381.0 – ∞)	141.8(113.0 – ∞)	141.8(113.0 – ∞)
Sub-adults					
Eleuthera	29	99.7(60.2 – 249.3)	210.7(103.0 – 8719.6)	84.8(61.1 – 133.5)	84.8(61.1 – 133.5)
Exuma	25	588.6(119.9 – ∞)	168.1(105.9 – 381.1)	168.1(105.9 – 381.1)	168.1(105.9 – 381.1)
Adults					
Eleuthera	30	–190.2(42052.3 – ∞)	–168.6(-606.8 – ∞)	–233.4(-3508.9 – ∞)	–233.4(-3508.9 – ∞)
Exuma	19	266.3(236.8 – ∞)	–317.4(326.9 – ∞)	–317.4(326.9 – ∞)	–317.4(326.9 – ∞)
Population 1					
Abaco	52	380.4(166.2 – ∞)	795.2(282.1 – ∞)	371.5(208.2 – ∞)	547.6(260.8 – ∞)
Andros	28	912.1(147.7 – ∞)	–1426.9(266.9 – ∞)	–770.4(377.2– ∞)	–770.4(377.2– ∞)
New Providence	8	67.5(18.8 – ∞)	67.5(18.8 – ∞)	67.5(18.8 – ∞)	67.5(18.8 – ∞)
Eleuthera	58	120.5.8(288.6 – ∞)	1249.8(354.1 – ∞)	797.6(322.3 – ∞)	553.9(280.0 – ∞)
Exuma	20	384.8(88.9 – ∞)	–542.9(205.4 – ∞)	–542.9(205.4 – ∞)	–542.9(205.4 – ∞)
Hail Mary	41	–3430.7(288.5 – ∞)	1335.5(295.5 – ∞)	2288.5(357.6 – ∞)	2288.5(357.6 – ∞)
Long Island	8	–32.0(-579.8 – ∞)	–32.0(-579.8 – ∞)	–32.0(-579.8 – ∞)	–32.0(-579.8 – ∞)
Great Inagua	36	912.6(196.2 – ∞)	–7214.6(349.5 – ∞)	–3249.2(423.3 – ∞)	–3249.2(423.3 – ∞)
Population 2					
Abaco	10	–17.6(–23.1 – ∞)	–17.6(–23.1 – ∞)	–17.6(–23.1 – ∞)	–17.6(–23.1 – ∞)

Andros	12	–61.1(1175.2 – ∞)	–59.9(156.4 – ∞)	–59.9(156.4 – ∞)	–59.9(156.4 – ∞)
New Providence	0	–	–	–	–
Eleuthera	2	NA	NA	NA	NA
Exuma	19	1089.5(112.7 – ∞)	164.8(87.5 – 1006.9)	164.8(87.5 – 1006.9)	164.8(87.5 – 1006.9)
Hail Mary	5	–9.1(–17.1 – ∞)	–9.1(–17.1 – ∞)	–9.1(–17.1 – ∞)	–9.1(–17.1 – ∞)
Long Island	12	–509.5(59.2 – ∞)	–73.8(–845.3 – ∞)	–73.8(–845.3 – ∞)	–73.8(–845.3 – ∞)
Great Inagua	5	–9.3(15.3 – ∞)	–9.3(15.3 – ∞)	–9.3(15.3 – ∞)	–9.3(15.3 – ∞)

Supplementary Table 8. LDNE effective population size, N_e estimates and 95% parametric confidence intervals for Nassau grouper for the subset dataset with critical allele frequencies ranging from 0.05 to 0.005. N_g denotes the numbers of genotypes used.

Location	N_g	LDNE N_e (95% CI)	LDNE N_e (95% CI)	LDNE N_e (95% CI)	LDNE N_e (95% CI)
		0.05	*0.02*	*0.01*	*0.005*
Subset Dataset					
Abaco	10	–43(–244.8 – ∞)	–43(–244.8 – ∞)	–43(–244.8 – ∞)	–43(–244.8 – ∞)
Eleuthera	10	–68.7(188.2 – ∞)	–68.7(188.2 – ∞)	–68.7(188.2 – ∞)	–68.7(188.2 – ∞)
Andros	10	–65.1(334.7 – ∞)	–65.1(334.7 – ∞)	–65.1(334.7 – ∞)	–65.1(334.7 – ∞)

Supplementary Table 9. M-ratio results for pre-bottleneck N_e thetas of 10,000 and 15,000 based on mutation rates of 1.5 x 10^{-4}, 5 x10^{-4}, 5.57 x 10^{-4} and 2 x 10^{-3}.

Location	M-ratio	M_c for Θ = 5.6	M_c for Θ = 8.4	M_c for Θ = 20	M_c for Θ = 22.28	M_c for Θ = 30	M_c for Θ = 33.42	M_c for Θ = 80	M_c for Θ = 120
		μ = 1.5 x 10^{-4}	μ = 1.5 x 10^{-4}	μ = 5 x 10^{-4}	μ = 5 x 10^{-4}	μ = 5.57 x 10^{-4}	μ = 5.57 x 10^{-4}	μ = 2 x 10^{-3}	μ = 2 x 10^{-3}
Abaco	0.564	0.586	0.595	0.601	0.598	0.596	0.593	0.563	0.541
Andros	0.601	0.563	0.568	0.565	0.563	0.555	0.552	0.510	0.480
New Providence	0.466	0.435	0.416	0.359	0.352	0.328	0.320	0.245	0.212
Eleuthera	0.512	0.576	0.584	0.587	0.585	0.580	0.576	0.542	0.516
Exuma	0.530	0.553	0.558	0.548	0.546	0.538	0.534	0.486	0.457
Hail Mary	0.560	0.570	0.578	0.576	0.574	0.568	0.566	0.527	0.498
Long Island	0.534	0.497	0.490	0.463	0.453	0.438	0.430	0.361	0.324
Great Inagua	0.568	0.555	0.561	0.555	0.553	0.542	0.539	0.492	0.462

Chapter III

Nassau grouper migration patterns during full moon suggest collapsed historic fish spawning aggregation and evidence of an undocumented aggregation

Manuscript published in Bulletin of Marine Science

Authors: Stump K, Dahlgren CP, Sherman KD, Knapp CR

Bull Mar Sci. 93(2):375–389. 2017
https://doi.org/10.5343/bms.2016.1042

research paper

Nassau grouper migration patterns during full moon suggest collapsed historic fish spawning aggregation and evidence of an undocumented aggregation

Kristine Stump [1]
Craig P Dahlgren [2, 3]
Krista D Sherman [1, 4]
Charles R Knapp [1 *]

[1] *Daniel P. Haerther Center for Conservation and Research, John G. Shedd Aquarium, 1200 South Lake Shore Drive, Chicago, Illinois 60605.*

[2] *Science and Policy, Bahamas National Trust (BNT), P.O. Box N-4105, Nassau, The Bahamas.*

[3] *Perry Institute for Marine Science, PO Box 435, 5356 Route 100, Waitsfield, Vermont 05673.*

[4] *Biosciences, University of Exeter, Stocker Road, Exeter, Devon EX4 4 QD, United Kingdom.*

* *Corresponding author email: <cknapp@sheddaquarium.org>, telephone: 312-692-2735.*

Date Submitted: 25 April, 2016.
Date Accepted: 7 November, 2016.
Available Online: 18 January, 2017.

ABSTRACT.—Many fish species migrate to form fish spawning aggregations. The temporal and spatial predictability of these migrations and spawning aggregation locations makes species vulnerable to overfishing, as the majority of an adult population within a large region may be harvested quickly with minimal effort. Historically, the Nassau grouper, *Epinephelus striatus* (Bloch, 1792), was an important fishery species throughout its range, but due to spawning aggregation overfishing, it is now rare in many reef ecosystems. In The Bahamas, stocks continue to decline despite the implementation of spawning aggregation protections. While more Nassau grouper spawning aggregations have been reported in The Bahamas than any other country, very few have been validated, and the dynamics of spawning migrations to and from these sites is poorly understood. Here, we used acoustic telemetry to describe, for the first time, Nassau grouper migrations along Andros Island, The Bahamas, which is bordered by one of the longest barrier reefs in the world. We report the likely extirpation of a historically important spawning aggregation and suggest Nassau grouper are migrating to a previously undocumented spawning location. Fish migrated in groups during the January 2015 full moon along the barrier reef shelf edge traveling roundtrip distances of 71.5–260.3 km [$\bar{x}$ = 164.5 (SD 65.7) km, n = 9]. These results are critical to assess the efficacy of current management strategies in The Bahamas. Thus far, all known spaning aggregations have been reported to the scientific community by fishers. Data from our study, however, suggest the presence of a potential spawning aggregation informed by passive telemetry and warrants further investigation.

In marine ecosystems, the formation of fish spawning aggregations—transient gatherings of a large number of individuals for reproductive purposes—is a widespread reproductive strategy (Domeier 2012). While the formation of spawning aggregations is reproductively advantageous for species distributed in reef habitats at low densities across large spatial scales, the behavior makes them particularly

vulnerable to overfishing (Coleman et al. 1996). Indeed, predictable aggregations of commercially-fished species allow fishers to maximize catch and profit with minimal effort (Sadovy and Domeier 2005, Erisman et al. 2012).

The ecological effects of overfishing spawning aggregations can be significant given that for many species, spawning aggregations can attract individuals from across broad regions (Sadovy de Mitcheson and Erisman 2012). Furthermore, the ostensible benefits for fish inhabiting no-take marine protected areas are negated if species reliant on spawning aggregations are fished outside protected area boundaries during migrations (Bolden 2000, Sadovy de Mitcheson and Erisman 2012). Overfishing spawning aggregations, therefore, can have profound impacts at local and regional scales across multiple jurisdictions and management regimes (Erisman et al. 2012, Green et al. 2015).

One of the best known examples of a fishery collapse from spawning aggregation overfishing is the Nassau grouper, *Epinephelus striatus* (Bloch, 1792), which was once one of the most important fishery species in the wider Caribbean (Sadovy and Eklund 1999). However, due to heavy exploitation, it is now rare in many coral reef ecosystems throughout its native range (Sadovy de Mitcheson et al. 2008), and the majority of its spawning aggregations no longer form (Sadovy and Eklund 1999, Sadovy de Mitcheson et al. 2013). As such, the Nassau grouper is classified as endangered by the International Union for the Conservation of Nature (IUCN) (Cornish and Eklund 2003) and is listed as threatened under the US Endangered Species Act (81 FR 42268, June 29, 2016).

Nassau grouper are important ecologically as predators on reef fish and invertebrates (e.g., Eggleston et al. 1997, Mumby et al. 2006, 2011), and are also economically and culturally significant. For example, Bahamians have fished grouper for centuries, and the fishery supports thousands of livelihoods, saturating the social fabric of the country (Cushion and Sullivan-Sealey 2008). Landings of Nassau grouper in The Bahamas were valued in excess of US $1.08 million in 2014 and represent a substantial contribution to revenue generated by fisheries (Sherman et al. 2016). The Bahamas is one of the few remaining countries where Nassau grouper populations still support many active spawning aggregations, but declines in abundance even within marine reserves, and the collapse of historical spawning aggregations have been noted (Sadovy and Eklund 1999, Sherman et al. 2016; C Dahlgren, Bahamas National Trust, unpubl data). Approximately 40 Nassau grouper spawning aggregations are reported in The Bahamas, elucidated through anecdotal accounts and local knowledge (Sadovy and Eklund 1999, Sherman et al. 2016). Very few, however, have been validated or studied scientifically, and thus the historic and current status of Nassau grouper spawning aggregations in The Bahamas is largely unknown.

Research focused on Nassau grouper spawning migrations has been limited in geographic scope throughout The Bahamas, with a narrow understanding of migrations and spawning aggregations from Andros Island. Andros, the largest island in The Bahamas, is bordered along the east coast by one of the longest barrier reefs in the world (Lopez et al. 2000), and is reported to support two Nassau grouper spawning aggregations. One spawning aggregation is reported off South Andros Island at Tinker Rocks, but no stock or migration information exists for this location except that fishing occurred historically (anonymous fisher, South Andros, pers comm). Two studies attempted to describe spawning stock sizes at the other Nassau grouper spawning aggregation located at High Cay, Andros (Fig. 1). A 1999–2001

hydroacoustic survey resulted in spawning stock size estimates between 9300 and 12,500 individuals (Ehrhardt and Deleveaux 2007). Those estimates, however, were not empirically validated in situ and starkly contrasted to diver surveys of the same area at the same time, which reported approximately 500 Nassau grouper during the January 1999 spawning period (Ray et al. 2000). Anecdotal accounts from local fishers also support the lower abundance estimates from the early 2000s (Park Warden, Bahamas National Trust, pers comm; anonymous fisher, South Andros, pers comm). There is no current information regarding stock assessments or migration patterns for the spawning aggregation at High Cay, Andros Island.

The lack of information concerning current stock assessments and associated migratory behavior at any Andros spawning aggregation is unfortunate, especially given that the Bahamas Department of Marine Resources implemented a targeted fishing closure of the High Cay aggregation for four 5-d periods around the full moons in November through February starting in 1998 (Ray et al. 2000). Since 2004, a national seasonal closure of Nassau grouper spawning aggregations has been implemented for up to 3 mo during the spawning season, though exact dates were announced annually and subjected to change. More recently in October 2015, The Fisheries Resources (Jurisdiction and Conservation) Act (http://laws.bahamas.gov.bs) was amended to include a fixed seasonal closure of the Nassau grouper fishery, making it illegal to take, land, process or sell Nassau grouper during the spawning season from 1 December through 28 February (Bahamas Ministry of Agriculture and Fisheries 2015). A similar strategy has been implemented by the Cayman Islands Department of the Environment, and several studies suggest that such management measures are effective for stock recovery and stability (e.g., Whaylen et al. 2007). However, in The Bahamas, Nassau grouper populations continue to decline since spawning aggregation protections were implemented (Cheung et al. 2013, Sherman et al. 2016), underscoring the need to better understand the extent, variance, and current state of spawning migrations within the country.

Revisiting the High Cay spawning aggregation from Andros Island offers an opportunity to assess the efficacy of a closed season management strategy since this aggregation was the first targeted spawning aggregation for closed-season management in 1998. The extensive reef system off Andros Island also offers the opportunity to study migratory behavior across a large system to better understand the ecology of the species and apply data to management. In the present study, we used diver surveys and acoustic telemetry to assess the current state of the High Cay spawning aggregation, and describe the migratory behavior of Nassau grouper within the Andros Island barrier reef system during a winter spawning period. Our specific objectives were to: (1) assess the current state of the High Cay Nassau grouper spawning aggregation, (2) describe timing of migrations with respect to the full moon, (3) determine if Nassau grouper migration pathways follow the Andros barrier reef shelf edge, and (4) estimate distance traveled and speed during migrations.

Methods

Site Description.—In total, 16 adult Nassau grouper were tracked passively from December 2014 through March 2015 along the approximately 217 km long barrier reef running parallel to the east coast of Andros Island, The Bahamas. The reef edge is characterized by steep drop-offs and dramatic underwater cliffs at depths from

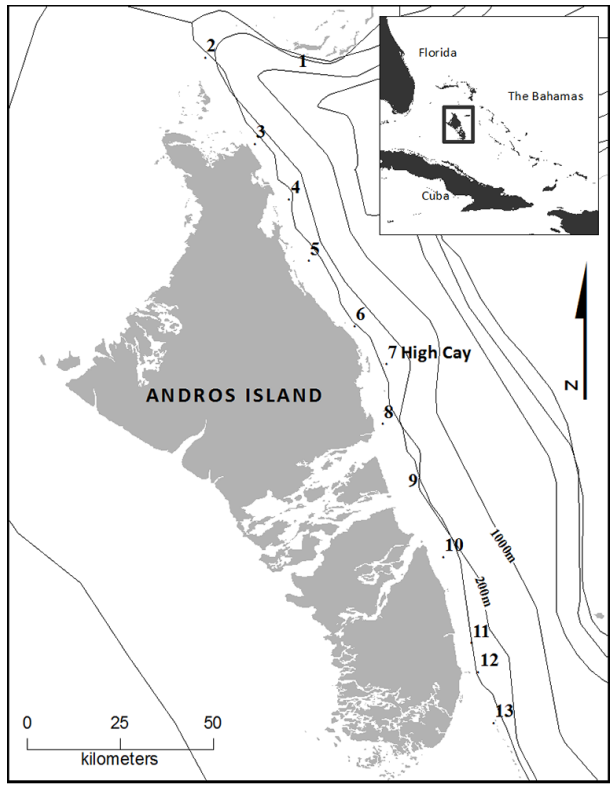


Figure 1. Map of study area (Andros, The Bahamas). Numbers indicate locations of the 13 acoustic receivers deployed along the barrier reef shelf edge. The two historically reported Nassau Grouper spawning aggregations, High Cay and Tinker Rocks, are located at receivers 7 and 11, respectively.

20–35 m before sloping steeply into the Tongue of the Ocean, a deep (1500–1800 m) cul-de-sac-shaped trench (Buchan 2000). We focused our fish capture efforts at the historical spawning aggregation reported near High Cay, a rocky outcrop about 3 km east of Andros (Fig. 1). The aggregation has been reported to form east of High Cay at depths of 25–45 m, along the barrier reef shelf edge (Ray et al. 2000). It is worth noting that a second aggregation site with similar bathymetric features is reported farther south near Tinker Rocks, though there is no scientific information regarding the past or present status of Nassau grouper spawning stocks at that area.

Acoustic Array.—Prior to deploying the acoustic array, the detection range for receivers was assessed at a representative reef site. During range testing, a receiver paired with a range test tag was deployed, and subsequent receivers were spaced at increasing distances from the first receiver and tag from 100 to 325 m away. Receivers were uploaded after 24 hrs. The number of range test tag detections on each receiver was compared to the number of detections by the receiver paired with the range test tag and reported as percent detections (Table 1).

Based on previous research demonstrating that Nassau grouper spawning migrations occur along shelf edges (Bolden 2000, Dahlgren et al. 2016a), an array of 13 Vemco™ VR2W acoustic monitoring receivers (Vemco, Ltd., Nova Scotia, Canada), was placed along the Andros barrier reef shelf edge in April 2014 (Fig. 1). Placement

Table 1. Results of VR2W acoustic receiver range testing at a representative reef site in The Bahamas.

Distance (m)	Detections (%)	Depth (m)
0	100.0	24.1
100	98.8	18.6
150	96.4	22.9
175	53.5	23.5
200	50.3	26.2
225	12.6	27.1
250	4.1	22.9
275	2.0	23.5
300	0.2	26.2
325	0.2	26.2

along the reef edge was predicted to detect any Nassau grouper migrating to and from the High Cay spawning aggregation. Receivers in the Andros array were spaced approximately 15 km apart, and the array extended from Chub Cay, Berry Islands, in the north to Grassy Cay, Andros, in the south (Fig. 1). Each VR2W was oriented facing upward, attached to a line approximately 3 m above the substrate, and each rig was anchored in place by two concrete blocks (Fig. 2). Floatation was provided by up to three styrofoam floats (depending on depth) and receivers were attached to the mooring line with four plastic ties (Fig. 2). Once a general deployment location was chosen, divers used a lift bag to slowly lower each rig onto an optimal location to minimize signal interference from nearby reef structure. Receivers remained in place through March 2015 when they were downloaded and redeployed for continued detection of Nassau grouper migrations during the 2015–2016 spawning season.

Fish Tagging.—Before deploying baited fish traps in December 2014, the research team dove at the reported High Cay spawning aggregation to confirm the presence of the aggregation. For 2 d before the full moon, divers reported seeing no Nassau grouper aggregation at the site described by Ray et al. (2000). This timeframe is consistent with peak numbers of aggregating fish at other sites in The Bahamas (Dahlgren et al. 2016a). Because exact spawning aggregation locations are known to shift slightly (Colin 1992, 2012), rotating teams of divers searched for aggregating Nassau grouper along the shelf edge (approximately 25–35 m depth) from 1 km south to 1 km north of the reported site.

To capture fish for tagging, baited fish traps were deployed within 100 m of the reported High Cay spawning aggregation. Trapped Nassau grouper were brought slowly to the surface to minimize barotrauma, and fish were kept in a 6745 L aerated, open-circulation live well on board the research vessel before processing. Each fish was transferred to a 100-L tricaine methanesulfonate (MS-222, 75 ppm) buffered seawater bath for anesthesia prior to transmitter surgery. While in the MS-222 bath, standard length (SL) and total length (TL) were recorded to the nearest 0.1 cm. Once anesthetized, each fish was weighed to the nearest 0.1 kg and then transferred to a 144-L aerated seawater bath for transmitter surgery. Each fish was held in a sling in the bath, ventral side up, and a small (2 cm) incision was made along the centerline, posterior to the pelvic fins. A Vemco™ V13 transmitter (13 × 36 mm, 6.5 g in water; Vemco, Ltd., Nova Scotia, Canada) with an estimated life span of 622 d was inserted

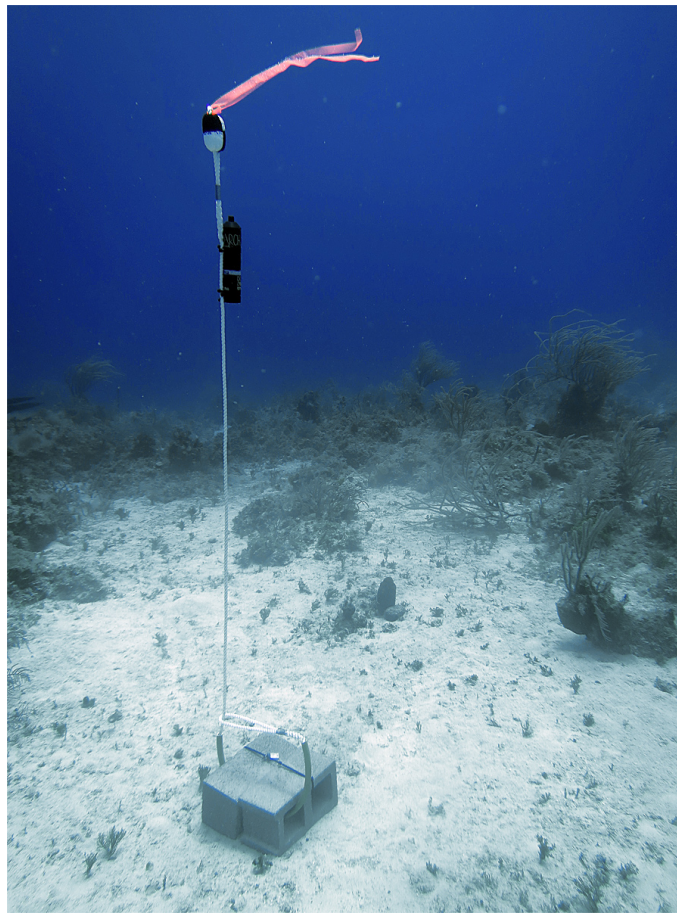


Figure 2. VR2W acoustic receiver deployed along the barrier reef shelf edge at approximately 25 m depth.

into the body cavity, and the incision closed with two to three cruciate sutures using Monosorb™ 3-0 absorbable monofilament. Following surgery, fish were transferred back to the 6745-L aerated, open-circulation live well to recover for 30–45 min prior to release. Once an individual maintained equilibrium and exhibited normal swimming behaviors, it was transferred to a mesh bag, brought to its original capture site, and released at depth by divers who monitored each individual for at least 1 min to ensure the fish continued to exhibit normal swimming behavior and was able to safely reach refuge.

Results

Migration Pathway and Timing.—Despite an exhaustive search, dive teams did not encounter an aggregation at the High Cay spawning aggregation during the full moon period of December 2014, when fish would be expected to arrive several days prior to the full moon based on other studies in The Bahamas (e.g., Colin 1992). Nevertheless, within 2 d prior to the full moon, 26 adult Nassau grouper [$\bar{x}$ = 62.7 (SD 3.7) cm TL] were captured in baited traps deployed within ≤100 m of the reported

Table 2. Summary of Nassau grouper, *Epinephelus striatus,* tagged at High Cay in December 2014. TL = total length. Number of detections indicates the total number of times an individual was detected across all receivers in the array during the January 2015 migration period. Northernmost detection corresponds to receiver numbers in Figure 1. Minimum distance traveled within the array is calculated from an individual's full set of detections and known distances between receivers.

Fish ID	TL (cm)	Number of detections	January 2015 migration	Northernmost detection	Swimming speed (km hr^{-1})	Minimum distance traveled within array (km)
152	66.0	11	North	4	1.91	103.9
157	59.2	15	North	4	1.08	105.6
159	62.0	11	North	4	1.52	103.9
161	64.1	32	North	2	2.02	260.3
162	59.0	15	North	2	1.30	154.7
164	63.0	6	North	1	1.30	95.2
166	61.4	15	North	3	1.73	208.0
170	67.5	25	North	3	2.34	208.0
174	63.0	10	North	2	2.02	241.3
192	63.0	8	South	–	1.67	71.5
151	61.0	17	No pattern	–	–	–
155	65.0	10	No pattern	–	–	–
153	52.5	0	Not detected	–	–	–
163	66.0	0	Not detected	–	–	–
168	63.0	0	Not detected	–	–	–
175	67.5	0	Not detected	–	–	–

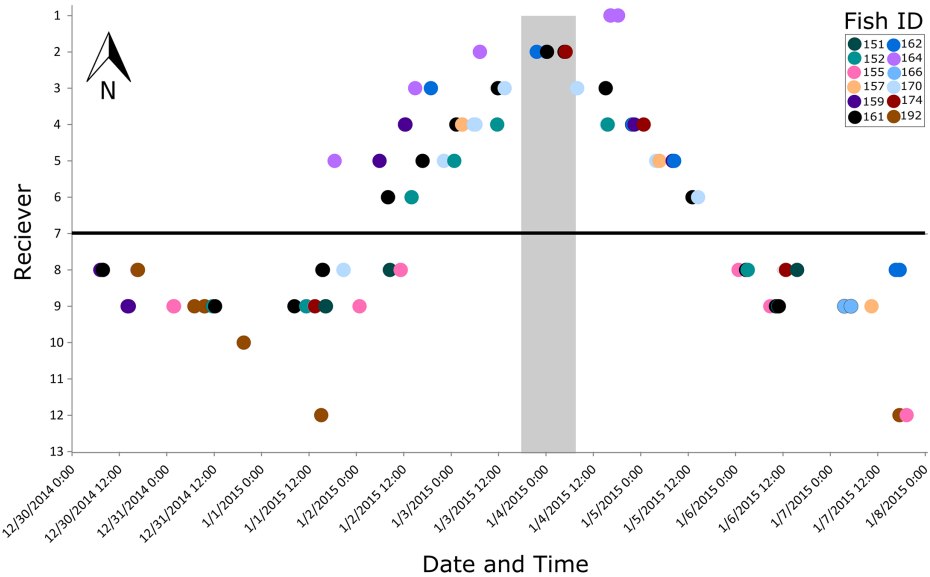


Figure 3. Detections in chronological order on each receiver for all grouper detected within the array during the migration period (4 d before to 4 d after the full moon; n = 12). Receivers are in order of increasing latitude (south to north), and numbers correspond to receiver numbers in Figure 1. The solid line along receiver 7 represents the location of the reported High Cay spawning aggregation. There are no data from this station because the receiver was lost. Note that distances between receivers are not uniform (see Fig. 1 for relative locations). The shaded area indicates the night of the January 2015 full moon.

spawning aggregation. Of those, 16 fish ranging in size from 52.5 to 67.5 cm TL [$\bar{x}$ = 62.7 (SD 3.7) cm TL] were surgically implanted with acoustic transmitters on 6 and 7 December, 2014 (Table 2). Following their release, none of the 16 grouper were detected for the remainder of the expected December full moon migratory period. However, 12 were detected moving north and south along the array during the expected full moon migratory period the following month in January 2015 (Table 2). Nine showed northward migrations, one made a southward journey, and two were detected, but exhibited no clear movement pattern (and thus removed from further analysis). As prior range testing produced reliable VR2W detection distances of up to 200 m (Table 1), Nassau grouper migratory movements were confirmed to occur along the edge of the Andros barrier reef tract. Detections for these 12 fish were synchronous with the full moon phase. That is, all of the grouper moving north (n = 9) began their directed northward movements 3 d before the night of the full moon, and all were detected at their northernmost point on the day of (n = 8) or the day after (n = 1) the full moon (Fig. 3). All grouper were once again detected at their southernmost point by 2 d after the full moon (Fig. 3). During northward and southward migrations, grouper were often detected within minutes of each other, suggesting synchronized group migrations to and from their destination along the barrier reef shelf edge.

Although nearly two thirds of all detections were recorded during the day, multiple detections (per night) over nine nights before and after the morning and evening astronomical twilight (approximately 05:30 and 19:00 hrs, respectively) were recorded for seven grouper. These detections (2–4 per fish) were limited to one receiver for

each fish. Six multiple nighttime detections (from five fish) were recorded over a 3–8 min time period and appeared consistent with fish moving past the receiver. Three multiple nighttime detections (from three fish) were recorded over a 1 hr 37 min to 4 hrs 23 min time period and may suggest a slowing or stopping individual. These prolonged periods between nighttime detections were recorded by two fish (161 and 162) on the night of the January 2015 full moon at receiver 2 in the north (Fig. 3), and the third fish (170) was detected over a prolonged period three nights after the full moon at receiver 9 in the south (Fig. 3).

When receivers were retrieved in March 2015, the VR2W at the reported High Cay spawning aggregation was missing. However, of the remaining 12 receivers along the barrier reef shelf, 10 detected tagged Nassau grouper during the spawning period. Many of the detections occurred sequentially in time along neighboring receivers, showing south to north movements (Fig. 3) in advance of, and just after, the January 2015 full moon. Surprisingly, however, these trajectories indicated that fish did not stop for an extended time near High Cay. Rather, all grouper migrating northward bypassed High Cay and continued toward the north end of Andros before the full moon and south toward central island latitudes after the night of the full moon (Fig. 3).

Migration Speed and Distance.—Of the 12 grouper detected within the array during the January 2015 migration, 10 were detected on sequential receivers, allowing for calculation of distance traveled within the array and migration speed (e.g., Starr et al. 2007, Rowell et al. 2015). The nine grouper migrating northward did so at an average speed of 1.69 (SD 0.41) km hr^{-1}. The one grouper traveling southward moved at 1.67 km hr^{-1}. One-way northward migrators averaged 104.0 (SD 18.7) km along the barrier reef shelf edge. Minimum roundtrip distances tracked within the array by northward migrating fish ranged from 95.2 to 260.3 km [$\bar{x}$ = 164.5 (SD 65.7) km, n = 9]. The southward migrating fish traveled a minimum distance of 71.5 km.

Discussion

Here, we provide evidence of a spawning aggregation collapse, as well as the first description of Nassau grouper spawning movements along the Andros barrier reef system, one of the world's largest reef tracts. Telemetry data show clear movement patterns tightly synchronized to the full moon. The likely extirpation of the historically-fished High Cay spawning aggregation is supported in part by telemetry data that demonstrate Nassau grouper migrations during the expected spawning period bypass the High Cay site en route to a more northern destination.

Despite an exhaustive search at the High Cay spawning aggregation site described both by local fishers and Ray et al. (2000), no Nassau grouper spawning aggregation was seen during the December 2014 spawning period. The diver-confirmed abundance estimate of the spawning stock size in 1999 was only 500, and the extirpation of even larger Nassau grouper spawning aggregations in a similar time span is not unprecedented in The Bahamas (Smith 1972, Colin 1992). However, adult Nassau grouper were indeed captured for the present study 1–2 d prior to the December full moon within 100 m of the reported aggregation site.

Prior to examining the telemetry data, the assumption was that dive teams missed the aggregation. However, detection data clearly demonstrate that in the following

month, grouper bypassed High Cay on northward migrations days before the night of the January full moon (Fig. 3). Potentially, the 16 grouper caught and tagged for the present study in December 2014 were all residents of the High Cay area. This is unlikely, however, as previously-collected survey data suggest extremely low densities for all *Epinephelus* spp. and *Mycteroperca* spp. in the Andros reef tract (<0.1 grouper per 100 m^2) (Dahlgren et al. 2016b). Additionally, only sexually-mature, adult Nassau grouper were captured in the baited traps. If the traps were sampling High Cay residents, we would expect to capture a range of sizes representative of the resident population instead of only adults.

In the absence of an observed spawning aggregation at High Cay, a plausible explanation for capturing so many adult Nassau grouper of sizes comparable to experienced migrators in The Bahamas (Dahlgren et al. 2016a) is that the grouper were captured during a northward migration to a different location. Telemetry data reveal that in January 2015, most grouper migrated >100 km along the barrier reef shelf edge to locations near the north end of the island (Fig. 3). Due to the loss of the receiver at High Cay, there are no detection data for that location. However, neighboring receivers to the north and south show that grouper were traveling in groups along the barrier reef shelf edge and passed through the High Cay area without slowing down.

Smith (1972) hypothesized that Nassau grouper migrated together along shelf edges to a spawning aggregation near Cat Cay in the northwest Bahamas, and suggested that there may be staging areas where grouper assemble before migrating en masse. This hypothesis was later corroborated by Colin (1992), who described migratory group movements along shelf edges south of Long Island, The Bahamas. In addition, telemetry data from the Exuma Cays to Long Island indicate that adult Nassau grouper tagged several kilometers apart passed receivers on shelf edges within hours of each other on the way to spawning aggregations in the days before the full moon (Dahlgren et al. 2016a). Similarly, telemetry data from the present study show that grouper migrating north along the Andros barrier reef shelf edge were frequently detected within minutes of each other along the length of the array. All but two of the grouper making northward migrations were first detected by receivers south of High Cay. In addition, on the return southward after the night of the full moon, all but one grouper with roundtrip detections swam past High Cay to receivers 8 and 9, 16.2 and 35.1 km south of High Cay, respectively (Fig. 3). Therefore, it is possible that baited traps used in our study captured adult Nassau grouper along their migration pathway rather than at their aggregation.

Our visual observations suggest that the historical Nassau grouper spawning aggregation at High Cay did not occur during the 2014–2015 spawning season. This is further supported by observations of fish migrating south to north along the Andros barrier reef past the High Cay location during the expected January 2015 spawning period. For several reasons, we believe the observed movement in January 2015 may represent a spawning migration to a location at the north end of the Andros barrier reef. First, none of the tagged grouper were detected at times other than during an expected migration period around the January full moon. During non-spawning periods, Nassau grouper are solitary reef dwellers with small home ranges on the order of 0.02 km^2 (Bolden 2001). If the home range of a tagged grouper overlapped with the detection range of one of our receivers, the individual would be detected over an extended time (e.g., Bolden 2000, Dahlgren et al. 2016a) and not just within 1 wk of the full moon. Second, based on a tested 200 m detection range for the receivers (Table 1),

long distance movements were confirmed to occur along the barrier reef edge. This movement is consistent with spawning migration movement pathways confirmed elsewhere in The Bahamas and in other parts of the Caribbean Sea (Bolden 2000, Starr et al. 2007, Colin 2012, Dahlgren et al. 2016a). Third, January 2015 movements were tightly synchronized to the full moon, as is characteristic of Nassau grouper spawning migrations in The Bahamas and elsewhere (e.g., Colin 2012). All grouper migrating north were first detected on their northward movement 3 d before the full moon, reached the northern peak of their journey on the full moon (n = 8) or the day after the full moon (n = 1), began southward movements immediately thereafter, and reached their southernmost detection point by 3 d after the night of the full moon (Fig. 3). Fourth, long-distance migratory fish moved in groups, a behavior that has been described previously (e.g., Colin 1992, Carter et al. 1994, Aguilar-Perera 2006). Finally, swimming speeds along the telemetry array during the migratory period averaged 1.69 (SD 0.41) km hr^{-1}, a value consistent with other telemetry studies (Starr et al. 2007, Dahlgren et al. 2016a), while six fish appeared to be migrating at night based on multiple detections made over a short nocturnal time period. Finally, nocturnal detections over a prolonged period up to 4 hrs and 23 min by two fish on the night of the full moon were recorded at receiver 2 in the northern extent of the array, suggesting that these fish stopped migrating during the time of expected spawning before making a migration back south. The relatively prolonged nocturnal detections at the southern extent of our detection range (receiver 9) for the remaining fish (170, Fig. 3) 3 d after the full moon (7 January, 2015) suggests that the fish reached the vicinity of its home range and stopped its migration.

If the grouper tagged in December 2014 and subsequently detected in January 2015 were indeed on spawning migrations during both periods, then the combination of capture and detection data suggest two migrations within one spawning season for 10 of the 16 tagged fish. Multiple migrations of individual Nassau grouper within one spawning season are common in other parts of the species' range, such as the Cayman Islands and Belize, where migratory distances between home reefs and spawning aggregations are generally 30 km or less (Semmens et al. 2006, Starr et al. 2007). In The Bahamas, however, where one-way spawning migrations have been shown to exceed 200 km (Bolden 2000, Dahlgren et al. 2016a), acoustic telemetry has revealed a rarity of multiple migrations by individual fish within one winter spawning period, with only a single fish observed to do so over several years of tracking multiple fish (Dahlgren et al. 2016a). Multiple intraseasonal migrations by an individual in The Bahamas have been recorded only when the second full moon after the autumnal equinox falls early in the spawning season (i.e., either the last week of November or first week of December) (Dahlgren et al. 2016a). When this occurs, during the first migration fish may not travel all the way to the intended spawning aggregation or return to home reefs in between full moons (Dahlgren et al. 2016a). The December 2014 full moon was indeed during the first week of the month and its timing may be related to the unexpected movement patterns. The relationship between migration distance, full moon timing, and intraseasonal migration occurrence warrants further investigation, as there may be intraspecific differences not only throughout the wider Caribbean region but also in The Bahamas.

Without in situ confirmation of an aggregation with concurrent spawning behavior, it is impossible to state with certainty that the January 2015 telemetry data reveal a previously unknown spawning aggregation in the northern Andros barrier reef,

or whether the observed migration is a response to the collapse of the historical High Cay spawning aggregation and subsequent exploratory movements in search of a non-existent spawning aggregation. The mechanisms by which fish learn migration routes and spawning aggregation locations are not fully understood. Some suggest learning through experienced migrators may play a key role, including sound production by experienced migrators guiding first-time spawners to spawning aggregations (e.g., Schärer et al. 2012, Rowell et al. 2015, but see Bernard et al. 2016). The consequences of harvesting the majority of adults from a spawning aggregation may lead to a dearth of experienced individuals to lead first-time cohorts (Sadovy de Mitcheson and Erisman 2012). Overharvest of adult Nassau grouper at High Cay, for example, could not only result in the extirpation of a season's spawning stock, but also prevent future migrators from learning both the pathway and destination. Recent evidence suggests, however, that aggregation recovery is possible. The US Virgin Islands, which experienced the loss of a Nassau grouper spawning aggregation due to overfishing, has experienced a slow reappearance of aggregations that may be the result of Nassau grouper mimicking the migration of yellowfin grouper, *Mycteroperca venenosa* (Linnaeus, 1758) (Nemeth et al. 2006, Kadison et al. 2009, Rowell et al. 2015). Therefore, if Nassau grouper are still in a region following the loss of a spawning aggregation, aggregation recovery may be possible at alternate locations.

Our results suggest both the collapse of a known spawning aggregation despite seasonal protection of the site, as well as a potentially unrecorded spawning aggregation located north of High Cay. Thus far, all known Nassau grouper spawning aggregations in The Bahamas have been reported and made known to the scientific community through fisher reports. If future visual observations confirm the presence of an unrecorded spawning aggregation, it will be the first time a spawning aggregation has been discovered using passive telemetry and underscores the versatility and importance of using this technology for monitoring and studying migratory fishes.

Acknowledgments

This work was supported by the John G Shedd Aquarium, including contributions from the Paul M Angell Foundation. Partial funding for C Dahlgren was provided by the Disney Conservation Fund's Reverse The Decline Initiative and a grant from the Atlantis Blue Project Foundation and the Moore Bahamas Foundation. Research was carried out under permits issued to K Stump and K Sherman by the Bahamas Department of Marine Resources (MAMR/FIS/17). Special thanks are given to L Knowles and the Bahamas National Trust. Thanks to J Rosauer for data processing. We are grateful to A Kough, R Gericke, and two anonymous reviewers for early reviews of this manuscript. We wish to acknowledge the many volunteers who participated in research cruises aboard the R/V Coral Reef II, as well as the research vessel's crew. All animal handling research protocols were followed in accordance with approval from Shedd Aquarium's internal and external research committee review process.

Literature Cited

Aguilar-Perera A. 2006. Disappearance of a Nassau grouper spawning aggregation off the southern Mexican Caribbean coast. Mar Ecol Prog Ser. 327:289–296. http://dx.doi.org/10.3354/meps327289

Bahamas Ministry of Agriculture and Fisheries. 2015. Fisheries resources (jurisdiction and conservation) (amendment) regulations. Available from: http://laws.bahamas.gov.bs/cms/images/LEGISLATION/SUBORDINATE/2015/2015-0071/FisheriesResourcesJurisdictionandConservationAmendmentRegulations2015_1.pdf

Bernard A, Feldheim KA, Nemeth R, Kadison E, Blondeau F, Semmens BX, Shivji MS. 2016. The ups and downs of coral reef fishes: the genetic characteristics of a formerly severely overfished but currently recovering Nassau grouper fish spawning aggregation. Coral Reefs. 35(1):273–284. http://dx.doi.org/10.1007/s00338-015-1370-3

Bolden SK. 2000. Long-distance movement of a Nassau grouper (*Epinephelus striatus*) to a spawning aggregation in the central Bahamas. Fish Bull. 98:642–645.

Bolden SK. 2001. Nassau Grouper (*Epinephelus striatus,* Pisces: Serranidae) movement in The Bahamas, as determined by ultrasonic telemetry. PhD dissertation. University of Miami, Miami.

Buchan KC. 2000. The Bahamas. Mar Pollut Bull. 41(1–6):94–111. http://dx.doi.org/10.1016/S0025-326X(00)00104-1

Carter J, Marrow GJ, Pryor V. 1994. Aspects of the ecology and reproduction of Nassau grouper, *Epinephelus striatus*, off the coast of Belize, Central America. Proc Gulf Caribb Fish Inst. 43:65–111.

Cheung WL, Sadovy de Mitcheson Y, Braynen MT, Gittens LG. 2013. Are the last remaining Nassau grouper *Epinephelus striatus* fisheries sustainable? Status quo in The Bahamas. Endanger Species Res. 20:27–39. http://dx.doi.org/10.3354/esr00472

Coleman FC, Koenig CC, Collins LA. 1996. Reproductive styles of shallow-water groupers (Pisces Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. Environ Biol Fishes. 47(2):129–141. http://dx.doi.org/10.1007/BF00005035

Colin PL. 1992. Reproduction of the Nassau grouper, *Epinephelus striatus* (Pisces: Serranidae) and its relationship to environmental conditions. Environ Biol Fishes. 34:357–377. http://dx.doi.org/10.1007/BF00004740

Colin PL. 2012. Timing and location of aggregation and spawning. *In*: Sadovy de Mitcheson Y, Colin PL, editors. Reef fish spawning aggregations: biology, research and management. London, UK: Springer. p. 117–158.

Cornish A, Eklund AM. 2003. *Epinephelus striatus*. The IUCN Red List of Threatened Species: e.T7862A12858266. Available from: http://www.iucnredlist.org/details/7862/0

Cushion N, Sullivan-Sealey K. 2008. Landings, effort and socio-economics of a small scale commercial fishery in The Bahamas. Proc Gulf Caribb Fish Inst. 60:162–166.

Dahlgren CP, Buch KL, Rechisky E, Hixon M. 2016a. Multi-year tracking of Nassau grouper (*Epinephelus striatus*) spawning migrations. Mar Coastal Fish. 8(1):522–535. http://dx.doi.org/10.1080/19425120.2016.1223233

Dahlgren C, Sherman K, Lang J, Kramer PR, Marks K. 2016b. Bahamas coral reef report card. Volume 1:2011–2013. 24 p.

Domeier ML. 2012. Revisiting spawning aggregations: definitions and challenges. *In*: Sadovy de Mitcheson Y, Colin PL, editors. Reef fish spawning aggregations: biology, research and management. London, UK: Springer. p. 1–21.

Eggleston DB, Lipcius RN, Grover JJ. 1997. Predator and shelter-size effects on coral reef fish and spiny lobster prey. Mar Ecol Prog Ser. 149:43–59. http://dx.doi.org/10.3354/meps149043

Ehrhardt NM, Deleveaux VKW. 2007. The Bahamas's Nassau grouper (*Epinephelus striatus*) fishery–two assessment methods applied to a data-deficient coastal population. Fish Res. 87:17–27. http://dx.doi.org/10.1016/j.fishres.2007.06.020

Erisman BE, Aburto-Oropeza O, Gonzalez-Abraham C, Mascareñas-Osorio I, Moreno-Baez M, Hastings PA. 2012. Spatio-temporal dynamics of a fish spawning aggregation and its fishery in the gulf of California. Sci Rep. 2. http://dx.doi.org/10.1038/srep00284

Green AL, Maypa AP, Almany GR, Rhodes KL, Weeks R, Abesamis RA, Gleason MG, Mumby PJ, White AT. 2015. Larval dispersal and movement patterns of coral reef fishes, and

implications for marine reserve network design. Biol Rev Camb Philos Soc. 90:1215–1247. http://dx.doi.org/10.1111/brv.12155

Kadison E, Nemeth RS, Blondeau J, Smith T, Calnan J. 2009. Nassau grouper (*Epinephelus striatus*) in St. Thomas, US Virgin Islands, with evidence for a spawning aggregation site recover. Proc Gulf Carib Fish Inst. 62:273–273.

Lopez JV, Peterson CL, Morales F, Brown L. 2000. Andros Island flora and fauna in the new millennium. Bahamas J Sci. 8(1):32–41.

Mumby PJ, Dahlgren CP, Harborne AR, Kappel CV, Micheli F, Brumbaugh DR, Holmes KE, Mendes JM, Broad K, Sanchirico JN, et al. 2006. Fishing, trophic cascades, and the process of grazing on coral reefs. Science. 311(5757):98–101. http://dx.doi.org/10.1126/science.1121129

Mumby PJ, Harborne AR, Brumbaugh DR. 2011. Grouper as a natural biocontrol of invasive lionfish. PLoS One. 6(6):e21510. http://dx.doi/10.1371/journal.pone.0021510

Nemeth RS, Kadison E, Herzlieb S, Blondeau J, Whiteman WA. 2006. Status of a yellowfin grouper (*Mycteroperca venenosa*) grouper spawning aggregation in the US Virgin Island with notes on other species. Proc Gulf Caribb Fish Inst. 57:543–558.

Ray GC, McCormick-Ray MG, Layman CA, Silliman BR. 2000. Investigations of Nassau grouper breeding aggregations at High Cay, Andros: implications for a conservation strategy. Final Report for the Department of Fisheries. Nassau, Bahamas. p. 13.

Rowell TJ, Nemeth RS, Schärer MT, Appeldoorn RS. 2015. Fish sound production and acoustic telemetry reveal behaviors and spatial patterns associated with spawning aggregations of two Caribbean groupers. Mar Ecol Prog Ser. 518:239–254. http://dx.doi.org/10.3354/meps11060

Sadovy Y, Domeier M. 2005. Are aggregation-fisheries sustainable? Reef fish fisheries as a case study. Coral Reefs. 24:254–262. http://dx.doi.org/10.1007/s00338-005-0474-6

Sadovy Y, Eklund AM. 1999. Synopsis of biological data on the Nassau grouper, *Epinephelus striatus* (Bloch, 1792), and the jewfish, *E. itajara* (Lichtenstein, 1822). NOAA Technical Report NMFS No. 146. NOAA/National Marine Fisheries Service, Seattle, WA. p. 65.

Sadovy de Mitcheson, Cornish A, Domeier M, Colin PL, Russel M, Lindeman KC. 2008. A global baseline for spawning aggregations of reef fishes. Conserv Biol. 22(5):1233–1244. http://dx.doi.org/10.1111/j.1523-1739.2008.01020.x

Sadovy de Mitcheson Y, Craig MT, Bertoncini AA, Carpenter KE, Cheung WWL, Choat JH, Cornish AS, Fennessy ST, Ferreira BP, Heemstra PC, et al. 2013. Fishing groupers towards extinction: a global assessment of threats and extinction risks in a billion dollar fishery. Fish Fish. 14:119–136. http://dx.doi.org/10.1111/j.1467-2979.2011.00455.x

Sadovy de Mitcheson Y, Erisman B. 2012. Fishery and biological implications of fishing spawning aggregations, and the social and economic importance of aggregating fishes. *In*: Sadovy de Mitcheson Y, Colin PL, editors. Reef fish spawning aggregations: biology, research and management. London, UK: Springer. p. 225–284.

Schärer MT, Rowell TJ, Nemeth MI, Appeldoorn RS. 2012. Sound production associated with reproductive behavior of Nassau grouper *Epinephelus striatus* at spawning aggregations. Endanger Species Res. 19:29–38. http://dx.doi.org/10.3354/esr00457

Semmens BX, Bush PG, Heppell SA, Johnson BC, McCoy CMR, Luke SKE, Pattengill-Semmens CV, Heppell S, Whaylen L. 2006. Defining the biogeography of a spawning aggregation to inform marine reserve planning and evaluation in the Cayman Islands. Proc Gulf Caribb Fish Inst. 59:638.

Sherman KD, Dahlgren CP, Stevens JR, Tyler CR. 2016. Integrating population biology into conservation management for endangered Nassau grouper *Epinephelus striatus*. Mar Ecol Prog Ser. 554:263–280. http://dx.doi.org/10.3354/meps11771

Smith CL. 1972. A spawning aggregation of Nassau grouper, *Epinephelus striatus* (Bloch). Trans Amer Fish Soc. 2:257–261. https://doi.org/10.1577/1548-8659(1972)101%3C257:ASAONG%3E2.0.CO;2

Starr RM, Sala E, Balelsteros E, Zabala M. 2007. Spatial dynamics of Nassau grouper *Epinephelus striatus* in a Caribbean atoll. Mar Ecol Prog Ser. 343:239–249. http://dx.doi.org/10.3354/meps06897

Whaylen L, Bush P, Johnson B, Luke KE, McCoy C, Heppell S, Semmes B, Boardman M. 2007. Aggregation dynamics and lessons learned from five years of monitoring a Nassau grouper (*Epinephelus striatus*) spawning aggregation in Little Cayman, Cayman Island, BWI. Proc Gulf Caribb Fish Inst. 59:413–422.

Chapter IV

RAD-seq and *in situ* monitoring of Nassau grouper reveal origins of aggregating fish

Manuscript in preparation

Authors: Sherman KD, Paris JR, Moore KA, King RA, Dahlgren CP, Stump K, Knowles LC, Stevens JR, Tyler CR

RAD-Seq analysis and *in situ* monitoring of Nassau grouper reveal fine-scale population structure and origins of aggregating fish

Krista D. Sherman[1,2,3], Josephine R. Paris[1,4], Karen A. Moore[1], R. Andrew King[1], Craig P. Dahlgren[3,5], Kristine Stump[2], Lindy C. Knowles[3], Jamie R. Stevens[1*] and Charles R. Tyler[1*]

[1]Biosciences, University of Exeter, Stocker Road, Exeter, Devon EX4 4QD, UK

[2]Daniel P. Haerther Center for Conservation and Research, John G. Shedd Aquarium, 1200 South Lakeshore Drive, Chicago, IL 60605, USA

[3]Science and Policy, Bahamas National Trust (BNT), P.O. Box N-4105, Nassau, Bahamas

[4]School of Life Sciences, University of Sussex, Brighton BN1 9RH, UK

[5]Perry Institute for Marine Science, P. O. Box 435, Waitsfield, VT 05673, USA

*Correspondence:

Dr. Jamie R. Stevens
J.R.Stevens@exeter.ac.uk

Professor Charles R. Tyler
C.R.Tyler@exeter.ac.uk

Abstract

A combined approach, integrating single-end restriction-site-associated DNA sequencing (RAD-seq) and acoustic telemetry was used for investigations of demographic structure, diversity and connectivity, and to determine the origins of Nassau grouper (*Epinephelus striatus*) utilising an active fish spawning aggregation (FSA) in the central Bahamas. RAD-seq analysis of 94 Nassau grouper sampled from nine locations in The Bahamas generated a total of 869,675 single nucleotide polymorphisms. Similar levels of heterozygosity (H_E=0.340–0.373) and nucleotide diversity (π=0.337–0.372) were found among sampled locations. Overall, Nassau grouper were not genetically differentiated (Global F_{ST}=0.0003, p=0.325), but significant genetic differentiation was detected between three population pairs. F_{ST} outlier tests identified a total of 181 loci under putative divergent selection associated with critical biological functions (for example immune/stress response and DNA damage repair). Hierarchical Discriminate Analysis of Principal Components (DAPC) revealed population sub-structuring, which was not evidenced by STRUCTURE analysis. This apparent pattern of sub-structuring was supported by explorations of gene flow and analyses of molecular variance (AMOVAs). Supporting acoustic telemetry data showed migrations of 22 % of tagged Nassau grouper (n=44) into the Exumas and identified the likely origins of five individuals, which migrated one-way distances of ~137 up to 176 km from an active fish spawning aggregation site in the central Bahamas to two sites in a no-take marine protected area (MPA) during the 2016–2017 spawning season. Together these findings provide novel information on the intraspecific population dynamics of Nassau grouper within The Bahamian archipelago and within an active FSA. Better understanding of the genetic population structure and spatial dynamics of Nassau grouper will contribute to the development of more effective management practices for the species.

Key Words: Effective population size, fish spawning aggregation, gene flow, population genomics, selection, single nucleotide polymorphisms, telemetry

Introduction

Approximately 25,000 of the species assessed worldwide have been classified as threatened and are at risk of extinction (IUCN 2017), including 12 % of grouper (Epinephelidae) species (Sadovy de Mitcheson et al. 2013). Changing this trajectory will require coordinated research and management approaches to develop a more robust understanding of population demographics at both small and large spatial scales. Current approaches include analyses of fisheries dependent and independent data for stock assessments (Thurstan et al. 2015; Egerton et al. 2017; Pauly and Zeller 2017), examination of habitat use and migratory behaviour (Austin 2007; Pittman et al. 2014; Aswani et al. 2015), modelling predicted responses to climate change (Cheung et al. 2012; Crozier and Hutchings 2014; Sunday et al. 2014), designating and monitoring marine protected areas (MPAs) or reserves (Hughes et al. 2007; Mumby and Harborne 2010; White 2015), establishing stocking programmes (Griffiths et al. 2011; Lorenzen et al. 2013; Abelson et al. 2016), recommending sustainable harvest regulations (Dann et al. 2013; Bozec et al. 2016; Hazen et al. 2016), and evaluating and incorporating the role of adaptation into fitness/survival (Eizaguirre and Baltazar-Soares 2014; Delmore et al. 2015; Calosi et al. 2016).

Nassau grouper (*Epinephelus striatus*, Bloch 1792) exemplify a species requiring immediate management due to their global critically endangered status, economic, cultural, and ecological significance (Sadovy de Mitcheson and Colin 2012; Carpenter et al. 2015; Sherman et al. 2016). Nassau grouper are inherently vulnerable to population declines because of their reproductive and life history traits (Coleman et al. 2000; Dahlgren and Eggelston 2001; Domeier 2012). More specifically, the predictable occurrence of lunar-associated migrations to known fish spawning aggregations (FSAs) for annual reproduction with conspecifics increases their susceptibility to overexploitation (Domeier 2012; Sadovy de Mitcheson and Erisman 2012; Cheung et al. 2013). As a result, many historic Nassau grouper FSAs have disappeared, and others currently exist with orders of magnitude fewer fish than they used to (Olsen and LaPlace 1979; Sadovy de Mitcheson et al. 2013).

Investigations of Nassau grouper FSAs have described the dynamic nature of these migrations – particularly, intraspecific differences in the timing of migrations (in relation to the winter solstice) and distances travelled to and

from FSAs (Smith 1972; Colin 1992; Bolden 2000; Starr et al. 2007; Dahlgren et al. 2016a). Additionally, adults are reported to exhibit site fidelity to specific FSAs (Heppell et al. 2009; Kadison et al. 2010; Dahlgren et al. 2016a) and tend to home to their respective reefs (Bolden 2000; Dahlgren et al. 2016a). However, FSAs are comprised of hundreds to thousands of individuals that migrate from multiple locations (Domeier 2012), and larvae are dispersed with ocean currents, which can facilitate genetic mixing of different stocks during the spawning season (Colin 1992; Molloy et al. 2012; Colin 2012). Indeed, previous research has demonstrated how variability in migratory behaviour can influence genetic connectivity (Dann et al. 2013; Delmore et al. 2015; Moore et al. 2017). The Bahamian archipelago represents an important area for Nassau grouper (Dahlgren et al. 2016b; Sherman et al. 2016), yet data on the current status of Nassau grouper, its spawning stocks, patterns of gene flow, population structure and the origins of migrating adults within the country are limited (Dahlgren et al. 2016a; Sherman et al. 2016, 2017; Stump et al. 2017). Such information is critical in order to effectively manage a species.

In recent decades, conservation biology has increasingly incorporated molecular tools to assist with fisheries management (Reiss et al. 2009; Flanagan et al. 2017). Several reviews have highlighted the advantages and limitations of molecular approaches for both marine and freshwater species (e.g. Davey et al. 2011; Selkoe et al. 2016; Lowe et al. 2017). In particular, highly connected marine species, characterised by weak genetic subdivision and large effective population sizes (N_e), often pose difficulties for population assignment and ascribing management units (MUs) (Allendorf et al. 2010), in comparison to freshwater species (e.g., Vidal and Marín 2011; Whiteley et al. 2011). For Nassau grouper, most assessments of genetic differentiation have been based on relatively small numbers of nuclear markers, i.e. mtDNA or microsatellites (Jackson et al. 2014; Bernard et al. 2016; Sherman et al. 2017), which may provide insufficient resolution to delineate fine-scale population structure.

Restriction-site-associated DNA sequencing (RAD-seq) can be used to identify and genotype large numbers of single nucleotide polymorphisms (SNPs) approximately evenly distributed throughout the genome across many individuals (Mardis 2008; Baird et al. 2008; Etter et al. 2011), for both model and non-model species (Hohenlohe et al. 2010; Davey et al. 2011; Ogden et al.

2013; Andrews et al. 2016). The exploration of SNPs in non-model organisms began nearly three decades ago and has grown tremendously in the ensuing years in conjunction with developments in sequencing technology and statistical processing tools (Davey et al. 2011; Seeb et al. 2011; Catchen et al. 2013). Increased coverage throughout the genome and sequencing depth using thousands of SNP markers have substantially improved the capacity to unravel genetic diversity and divergence in species with both strong (Perrier et al. 2013) and weak (Larson et al. 2014; Benestan et al. 2015) population structure. For example, Larson et al. (2014) used 10,944 SNPs to reveal genetic population structuring in chinook salmon (*Oncorhynchus tshawytscha*) and applied genomic data to more reliably assign fish to regions that they originated from. More recently, Benestan et al. (2015) also used RAD-seq to reveal previously undetected regional and fine-scale genetic structure in American lobster (*Homarus americanus*) and identified locations of origin for ~81% of the 586 lobsters used in the study.

While a few thousand SNPs have been identified and utilised in Caribbean-wide assessments of genetic subdivision for Nassau grouper (Jackson et al. 2014) to date, such an approach has not been used to explore fine-scale patterns of genetic variation in The Bahamas. Better characterization of the spatiotemporal population dynamics of Nassau grouper through genome-wide assessments of genetic diversity and *in situ* investigations of spawning migration patterns are critical to develop effective conservation management strategies. In this study, we combined RAD-seq and acoustic telemetry to provide more detailed insight into the spatial population dynamics and intraspecific genetic population structure of Nassau grouper in The Bahamas. Specific objectives were to 1) use SNPs to resolve fine-scale genetic variation throughout The Bahamas, 2) investigate the role of selection on genetic population structure, 3) examine patterns of contemporary gene flow within The Bahamas and, 4) use acoustic telemetry to determine the origins of Nassau grouper migrating to Hail Mary, an active spawning aggregation in the central Bahamas.

Methods

Sampling, DNA Extraction and Quantification for RAD-seq

Nassau grouper fin clip samples (n=94) were collected according to methods described by Sherman et al. (2017). Samples for RAD-seq analysis originated from eight islands (Abaco, Andros, Eleuthera, Exuma, Great Inagua, Long Island, New Providence and Ragged Island) and the Hail Mary FSA in The Bahamas. Fin clips were preserved in 95–100 % ethanol prior to genomic DNA extraction. Genomic DNA was isolated from fin clip tissues (n=96) using the Qiagen DNeasy Blood and Tissue Kit® according to the manufacturer's instructions, quality assessed via agarose gel electrophoresis, and quantified using the dsDNA BR Qubit Quant-iT assay (Invitrogen). DNA samples were prepared for RAD library development by normalising concentrations of each sample to 50 ng/µl.

RAD Library Development and Sequencing

RAD-seq libraries for 96 Nassau grouper were prepared by the Exeter Sequencing Service (University of Exeter, United Kingdom) using Illumina Nextera XT barcodes. A total of 400 ng DNA was sheared to an average size of 1,000 bp using a Covaris E220 sonicator optimised for a size range 800-1,000 bp under the following fragmentation conditions: time 45s, peak incident power 175, duty factor 2 %, cycles per burst 200, temperature 8 °C and volume 50 µl. Fragmentation of eight samples was checked using a DNA 1000 screentape (Agilent). The NEBNext ultra II DNA library preparation kit was used for end-repair, A-tailing, and to ligate P2 adapters. Reactions were purified using AmpureXP beads (Beckmann Coulter). P2 adapted-DNA was digested using the restriction enzyme *SbfI* (New England Biolabs) at 37 ˚C for four hours and purified using AmpureXP beads to avoid enzyme denaturation. Phased P1 adapters were ligated to the digested fragments and unligated adapters removed using AMPureXP magnetic beads. The P1 adapter was biotinylated at the 5'end of the top strand to enable capture with streptavidin beads. After washing away fragments not bound to the P1 adapter, the DNA was amplified by PCR to add NexteraXT multiplexing barcodes and flow cell attachment regions to complete the library preparation. Library quality and quantity was

assessed using DNA screentapes. Equimolar pooling of the libraries was undertaken before size selection of libraries 450–800 bp (inserts ~320–680 bp). The size-selected pool was quantified by qPCR. Libraries were denatured and diluted to 9 pM with 5 % PhiX spike-in and 100 bp single-end sequenced on two lanes (48 individuals per lane) of Illumina HiSeq 2500 using v.2 SBS Rapid reagents.

SNP Discovery and Genotyping

FastQC was used to inspect the quality of raw sequence reads (Andrews 2010). *Stacks* v. 1.42 (Catchen et al. 2011, 2013) was used for RAD-seq data processing and population genomic analyses. Raw sequence data were de-multiplexed, cleaned, adapters removed, low quality reads discarded (i.e. ≤10) and truncated to 96 bp using the program *process_radtags*. Following recommendations by Paris et al. (2017), *de novo* assembly parameters were optimised (*m*: 3–7*, M*: 1–7 and *n*: 2–4; see Supplementary Fig. 1)

The *denovo_map* pipeline was re-run using optimal parameters of m5, M3 and n3. Two samples — one from Andros (AN026) and one from Hail Mary (LI239) — were removed from subsequent analyses due to low coverage (Supplementary Tables 1–2). The program, *cstacks* was used to rebuild a *catalog* of loci using individuals with coverage depths of ≥15x (n=59) to reduce the likelihood of genotyping recurrent sequencing error. Loci from all individuals were then matched back to the *catalog* using the *sstacks* program to generate genotypes. Genetic population statistics were computed using the *populations* program. Under this component of *Stacks*, for a locus to be retained it had to occur in at least five out of nine (~56 %) populations to obtain SNP data common across 80 % of individuals within all sampled locations. We set the minor allele frequency to 10 % and the maximum observed heterozygosity to 70 %. This resulted in a final dataset consisting of 10,031 polymorphic loci, which was exported as a GENEPOP formatted file (Raymond and Rousset 1995) and further conversions for data analysis were implemented using PGD Spider v. 2.0.1.0 (Liscjher and Excoffier 2012) or Formatomatic v. 0.8.1 (Manoukis 2007).

Population Diversity, Differentiation, Structure and Effective Population Size

For population genetic assessments, conformity to Hardy-Weinberg equilibrium was performed in GENEPOP v.4.2 (Rousset 2008). To detect Type I errors in both tests, the false discovery rate (FDR) correction was employed (Storey and Tibshirani 2003). To address linkage disequilibrium and to account for potential ascertainment bias, only a single random SNP was selected using the *(--write_single_snp* function) from each RAD-tag. Standard diversity measures including allele frequencies, expected and observed heterozygosity (H_E and H_O), F_{IS} and numbers of polymorphic loci were computed in GenoDive v. 2.0b27 (Meirmans and Van Tienderen 2004) using 10,000 iterations of the data. Global and pairwise comparisons of F_{ST} (Weir and Cockerham 1984) were also computed using GenoDive v. 2.0b27.

For analyses sensitive to outlier loci, a neutral dataset containing all polymorphisms (26,885 loci) was used (see Results). Two methods were used for assessment of genetic population structure of Nassau grouper. The first method consisted of performing hierarchical DAPCs using the *adegenet* package in R v. 1.0.143 (Jombart 2008; Jombart et al. 2010). The number of principal components employed to create the initial DAPC was based on inspections of the optimal α-score and cross-validation outputs (Jombart et al. 2010). To avoid over-fitting the data (which would lead to erroneous genetic clusters), the number of principal components retained for analysis was based on the lower of the two outputs. Subsequent DAPCs were constructed using the optimal α-score and three discriminant functions were used for each DAPC analysis. Secondly, we used STRUCTURE analysis (Pritchard et al. 2000) implementing a pre- and post-burn-in of 10,000 and 10,000 Monte Carlo Markov Chain iterations respectively over 10 independent runs for inferred populations, *K,* ranging from 1–10 under the automated program StrAuto (Chhatre & Emerson 2017). The Evanno delta *K* (Δ*K*) statistic (Evanno 2005) was calculated using the web-based Structure Harvester v. 0.6.94 programme (Earl and vonHoldt 2012) and used to identify the most likely genetic clusters of the dataset. Visualisation of STRUCTURE runs was performed using the *pophelper* package in R (Francis 2017).

Results of the sub-structure suggested by DAPC analyses were used to develop and test hypotheses for analyses of molecular variance (AMOVAs). Based on the three groupings identified by DAPC analysis of the full SNP dataset (Fig. 3a), data were partitioned into three groups for subsequent

AMOVAs. The infinite alleles model (IAM) in GenoDive was selected to perform three AMOVAs based on 10,000 iterations of the neutral SNP dataset to test whether genetic variation was greater between 1) The Bahamas and Exuma, 2) The Bahamas and Long Island, or 3) The Bahamas versus Exuma and Long Island. Finally, NeEstimator v. 2.1 (Do et al. 2014) was employed to produce estimates of effective population size (N_e) for each location. N_e was calculated for the full and neutral datasets using the linkage disequilibrium method, assuming a random mating model with a critical allele frequency of 0.01.

Identifying Loci under Selection

We employed two F_{ST} outlier methods to screen for loci under selection. First, Bayescan v. 2.1 (Foll and Gaggioti 2008) was run under prior odds of 10, 100, and 1,000 respectively with a burn-in of 50,000 and a thinning interval of 10 for 100,000 Markov chain Monte Carlo (MCMC) iterations. Secondly, 100,000 simulations of the data were permuted in Lositan (Beaumont and Nichols 1996; Antao et al. 2008) with confidence intervals of 0.995, FDR of 0.05 under the IAM. A neutral mean F_{ST} was run prior to the main analysis in order to remove false positives and mean F_{ST} was forced to exclude the first set of outliers. To determine whether loci under selection may be influencing genetic structure, DAPCs were constructed with loci detected as potentially under positive and balancing selection and were compared to the genetic structure identified using only selectively neutral loci. Finally, the basic aligner search tool (BLAST, https://blast.ncbi.nlm.nih.gov/Blast.cgi) was used to identify functional attributes of the list of candidate loci under positive and balancing selection.

Gene Flow

Ten random subsets of 100 neutral SNPs (excluding individuals from Hail Mary) were employed to examine patterns of contemporary gene flow across The Bahamas using BayesAss v. 3.0.4 (Wilson and Rannala 2003). The programme operates using a Bayesian framework to estimate the proportion of first generation migrants in each putative population (i.e., island or sample location). BayesAss was implemented with a sampling frequency of 1,000 and burn-in of 10^6 for 10^7 iterations. Mean values from the 10 runs were calculated and used to create gene flow diagrams using the circlize package (Gu 2014) in R.

During December 2014 – December 2016, Nassau grouper collected around the Exumas, Long Island and the Hail Mary FSA, were externally tagged (n=103) using colour-coded Floy™ tags and appropriately sized fish, i.e. ≥54 cm total length, TL (n=44) were also surgically implanted with Vemco™ V13 or V13P (13 mm x 36 mm, 6.5 g in water; Vemco, Ltd., Nova Scotia, Canada) acoustic transmitters and released as per procedures outlined by Stump et al. (2017). Eleven acoustic receivers (VR2Ws) stationed in the Exumas (n=3), Long Island (n=3) and at the Hail Mary FSA (n=5) were used to track and record data on Nassau grouper migrations to and from the Hail Mary FSA over consecutive spawning seasons between December 2014–January 2017 (Figs. 1–2).

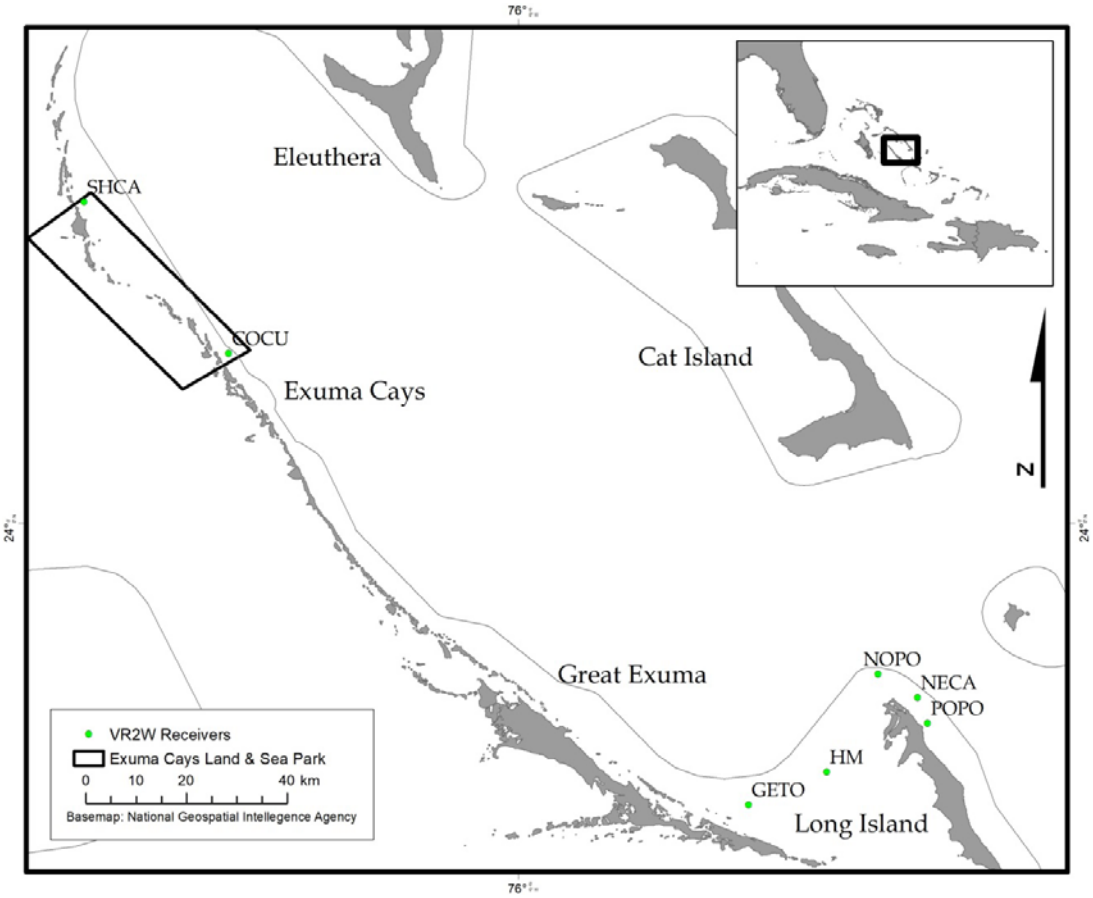


Figure 1. Map of acoustic receivers (VR2Ws) [denoted by green circles] deployed around the Exumas and Long Island, Bahamas. Receiver codes correspond to: SCHA = Shroud Cay, COCU = Conch Cut, GETO =

Georgetown, HM = Hail Mary, NOPO = North Point, NECA = Newton's Cay and POPO = Poseidon's Point. SHCA and COCU are located within the boundaries no-take Exuma Cays Land and Sea Park (rectangular shape outlined in black). The inset map shows the Exuma Sound study area (black box) in relation to the rest of The Bahamas, Florida and the Caribbean.

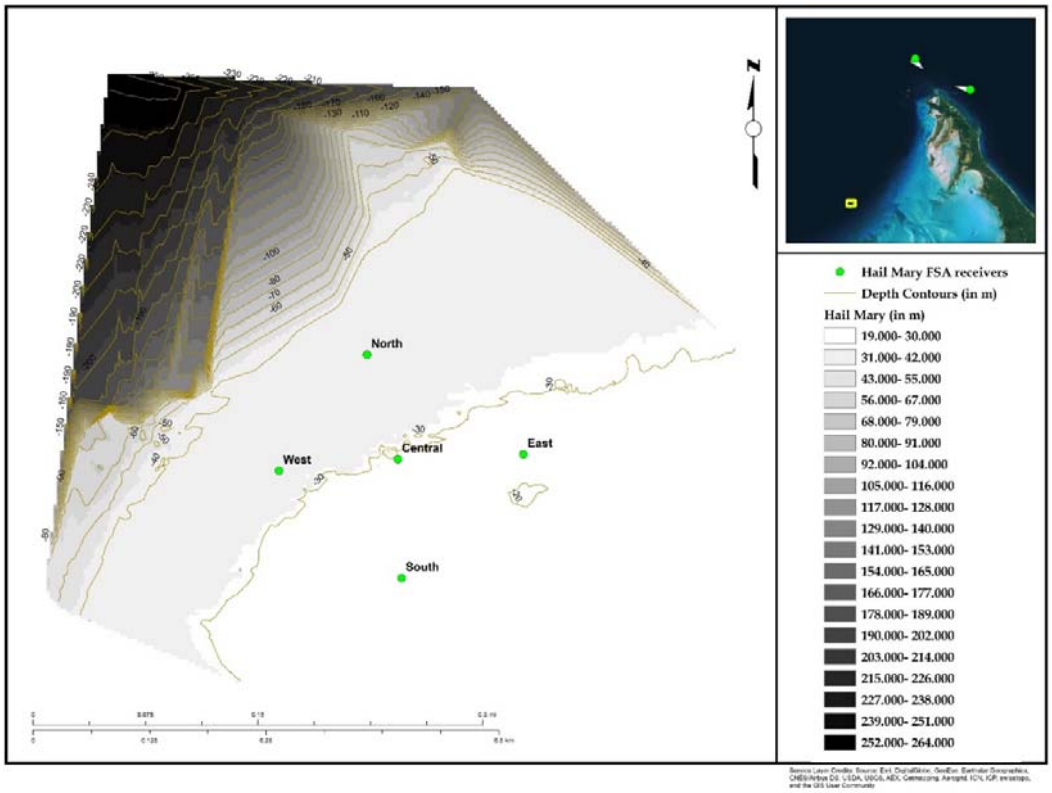


Figure 2. Bathymetry map of the Hail Mary FSA. Green circles denote the location of the five acoustic bottom monitors (VR2Ws) deployed within a 1 km^2 area at the site. The inset map depicts the location of Hail Mary (yellow box) in relation to two other reported Nassau grouper FSAs located around the northern part of Long Island, Bahamas, where the North Point (NOPO) and Newton's Cay (NECA) receivers (VR2Ws) have been placed.

Results

SNP Discovery and Genotyping

RAD-seq of 96 Nassau grouper produced a total of 159,195,960 raw RAD-tags with an average of 1,658,291.25 (±671,515.34 SD) RAD-tags across

all samples (Supplementary Table 1). The number of RAD-tags generated for individual Nassau grouper ranged from 272,483 (LI239) to 3,653,394 (LI227). Following filtering and quality control, 114,174,309 RAD-tags (~72 %) were retained, with a mean of 1,189,315.72 (±478,376.18 SD) RAD-tags across samples (Supplementary Table 1). After parameter optimisation, a total of 5,139,297 assembled loci (min=20,298; max=63,594), 610,951 polymorphic loci (min=1,382; max=8,687), 869,675 polymorphic SNPs (min=2,489; max=12,139), and 78,615 fixed SNPs (min=19; max=8,216) were obtained (Supplementary Table 2). Average numbers of assembled loci, polymorphic loci, SNPs and fixed SNPs were 54,673.37 (±8,316.81 SD), 6,499.48 (±1,695.22 SD), 9,251.86 (±2,172.84 SD), and 836.33 (±1,253.21 SD) respectively (Supplementary Table 2). Approximately 35 % of loci were polymorphic and contained an average of 1.3 SNPs per locus. Numbers of heterozygous or fixed SNPs ranged from 19 to 8,216 (Supplementary Table 2). After filtering for 5/9 (56 %) populations, common across 80 % of individuals within all sampled locations, the final dataset contained 28,636 assembled loci, of which 10,031 were polymorphic, containing a total of 13,241 SNPs.

Genetic Diversity, Differentiation and Structure

Assessments of genetic diversity, differentiation and population structure were based on 10,031 polymorphic loci containing 13,241 SNPs. A total of 262 loci deviated from HWE after FDR correction ($p<0.05$). However, because all populations conformed to HWE, no loci were discarded. Measures of genetic diversity and allele polymorphism were generally consistent across sample locations (Table 1). Mean genome-wide diversity (based on all loci) across all locations was H_E=0.3627 and H_O=0.332. Heterozygosity, H_E ranged from 0.340–0.373; Table 1). Observed heterozygosity, H_O was lowest in Abaco (0.284) and highest in Exuma (0.363; Table 1). Coefficients of inbreeding were highest in Abaco (F_{IS} = 0.127, G_{IS} = 0.165) and lowest in Exuma (F_{IS} = 0.024, G_{IS} 0.027; Table 1). Generally, neutral loci exhibited reduced levels of H_E, H_O, F_{IS}, G_{IS}, nucleotide diversity (π), and allele polymorphism, compared with the full dataset (all loci), positive and balancing loci (Table 1).

Table 1. Summary indices of genetic diversity per location based on all loci (n=10,031), neutral loci (n=26,885), positive loci (n=181) and balancing loci (n=212).

All loci Location	Location Code	N	No. Alleles	Effective No. Alleles	H_O	H_E	H_t	F_{IS}	G_{IS}	π
Abaco	AB	10	1.95	1.53	0.284	0.340	0.340	0.127	0.165	0.337
Andros	AN	9	1.96	1.56	0.323	0.355	0.355	0.069	0.089	0.353
Eleuthera	EL	16	2.00	1.59	0.335	0.362	0.362	0.067	0.075	0.361
Exuma	EX	11	1.99	1.60	0.363	0.373	0.373	0.024	0.027	0.372
Great Inagua	GI	6	1.90	1.54	0.319	0.354	0.354	0.068	0.099	0.350
Hail Mary FSA	HM	21	2.00	1.60	0.346	0.367	0.367	0.053	0.056	0.366
Long Island	LI	10	1.98	1.59	0.344	0.368	0.368	0.054	0.067	0.367
New Providence	NP	6	1.90	1.54	0.321	0.356	0.356	0.067	0.098	0.353
Ragged Island	RI	5	1.86	1.53	0.325	0.355	0.355	0.052	0.084	0.351
Neutral loci										
Abaco	AB	10	1.38	1.15	0.091	0.101	0.101	0.026	0.097	0.100
Andros	AN	9	1.43	1.17	0.111	0.115	0.115	0.013	0.042	0.115
Eleuthera	EL	16	1.55	1.19	0.118	0.123	0.123	0.016	0.038	0.123
Exuma	EX	11	1.54	1.20	0.132	0.132	0.132	0.004	0.003	0.132
Great Inagua	GI	6	1.35	1.17	0.108	0.115	0.115	0.015	0.061	0.114
Hail Mary FSA	HM	21	1.64	1.19	0.124	0.128	0.128	0.015	0.029	0.128
Long Island	LI	10	1.49	1.19	0.124	0.129	0.129	0.013	0.033	0.128
New Providence	NP	6	1.37	1.17	0.114	0.120	0.120	0.014	0.053	0.119
Ragged Island	RI	5	1.33	1.17	0.112	0.117	0.117	0.009	0.041	0.117
Positive loci										
Abaco	AB	10	1.81	1.44	0.227	0.285	0.285	0.119	0.204	0.281
Andros	AN	9	1.96	1.56	0.287	0.357	0.357	0.143	0.196	0.353
Eleuthera	EL	16	1.99	1.62	0.319	0.378	0.378	0.132	0.156	0.375
Exuma	EX	11	1.97	1.64	0.362	0.382	0.382	0.045	0.052	0.381
Great Inagua	GI	6	1.78	1.46	0.245	0.303	0.303	0.120	0.191	0.297
Hail Mary FSA	HM	21	1.99	1.64	0.332	0.379	0.379	0.119	0.125	0.378
Long Island	LI	10	1.95	1.60	0.312	0.367	0.367	0.121	0.151	0.364
New Providence	NP	6	1.82	1.47	0.277	0.309	0.309	0.058	0.103	0.305
Ragged Island	RI	5	1.63	1.40	0.222	0.269	0.269	0.088	0.177	0.263
Balancing loci										
Abaco	AB	10	1.52	0.30	0.342	0.342	0.34	0.104	0.119	0.339
Andros	AN	9	1.55	0.34	0.353	0.353	0.35	0.025	0.024	0.352
Eleuthera	EL	16	1.53	0.33	0.338	0.338	0.34	0.010	0.016	0.338
Exuma	EX	11	1.56	0.34	0.357	0.357	0.36	0.039	0.041	0.356
Great Inagua	GI	6	1.58	0.35	0.391	0.391	0.39	0.083	0.097	0.387
Hail Mary FSA	HM	21	1.54	0.32	0.340	0.340	0.34	0.057	0.052	0.339
Long Island	LI	10	1.56	0.32	0.362	0.362	0.36	0.093	0.108	0.360
New Providence	NP	6	1.58	0.35	0.386	0.386	0.39	0.067	0.091	0.383
Ragged Island	RI	5	1.58	0.40	0.397	0.397	0.4	0.002	0.003	0.397

Global F_{ST} of all loci was low and non-significant (Global F_{ST} = 0.0003, p = 0.325). Genetic differentiation based on pairwise F_{ST} values from these loci ranged from -0.003–0.003 and was greatest between Exuma and Great Inagua (0.003), and Hail Mary and Ragged Island (0.003). Pairwise F_{ST} values were significant (p <0.05) between only three population pairs (Table 2). No significant differences were found with either neutral (Global F_{ST} = -0.001, p = 0.976) or balancing loci (Global F_{ST} = -.0.053, p = 1.00). Conversely, pairwise differences using positive loci were mostly significant (Global F_{ST} = 0.124, p = 0.00), with estimates ranging from 0.004–0.353 (Table 2).

Table 2. Pairwise genetic differentiation, F_{ST} based on all loci (n=10,031), neutral loci (n=26,885), positive loci (n=181) and balancing loci (n=212). Significant F_{ST} values appear in bold.

All loci									
F_{ST}	Abaco	Andros	Eleuthera	Exuma	Great Inagua	Hail Mary	Long Island	New Providence	Ragged Island
Abaco	--	-0.002	0.001	0.003	0.001	0.002	0.001	0	-0.003
Andros	-0.002	--	-0.002	0.001	-0.001	0	-0.003	-0.003	-0.001
Eleuthera	0.001	-0.002	--	0.001	0.001	0	0	-0.001	0
Exuma	0.003	0.001	0.001	--	**0.003**	0	-0.001	**0.002**	0.002
Great Inagua	0.001	-0.001	0.001	0.003	--	0.001	0.001	0.002	0.002
Hail Mary	0.002	0	0	0	0.001	--	0	0.002	**0.003**
Long Island	0.001	-0.003	0	-0.001	0.001	0	--	0.001	0.001
New Providence	0	-0.003	-0.001	0.002	0.002	0.002	0.001	--	-0.002
Ragged Island	-0.003	-0.001	0	0.002	0.002	0.003	0.001	-0.002	--
Neutral loci									
	Abaco	Andros	Eleuthera	Exuma	Great Inagua	Hail Mary	Long Island	New Providence	Ragged Island
Abaco	--	0.000	-0.001	0.002	0.000	0.000	0.000	0.000	-0.003
Andros	0.000	--	-0.001	0.001	-0.001	0.000	-0.001	-0.002	0.000
Eleuthera	-0.001	-0.001	--	0.001	0.000	0.000	0.000	-0.002	-0.002
Exuma	0.002	0.001	0.001	--	0.000	0.001	-0.001	-0.001	-0.002
Great Inagua	0.000	-0.001	0.000	0.000	--	-0.002	-0.002	-0.002	-0.001
Hail Mary	0.000	0.000	0.000	0.001	-0.002	--	-0.001	-0.001	-0.001
Long Island	0.000	-0.001	0.000	-0.001	-0.002	-0.001	--	-0.001	-0.002
New Providence	0.000	-0.002	-0.002	-0.001	-0.002	-0.001	-0.001	--	-0.003
Ragged Island	-0.003	0.000	-0.002	-0.002	-0.001	-0.001	-0.002	-0.003	--
Positive loci									
	Abaco	Andros	Eleuthera	Exuma	Great Inagua	Hail Mary	Long Island	New Providence	Ragged Island
Abaco	--	**0.071**	**0.085**	**0.087**	**0.226**	**0.073**	**0.115**	**0.200**	**0.325**
Andros	**0.071**	--	**0.025**	**0.047**	**0.176**	0.012	**0.039**	**0.149**	**0.247**

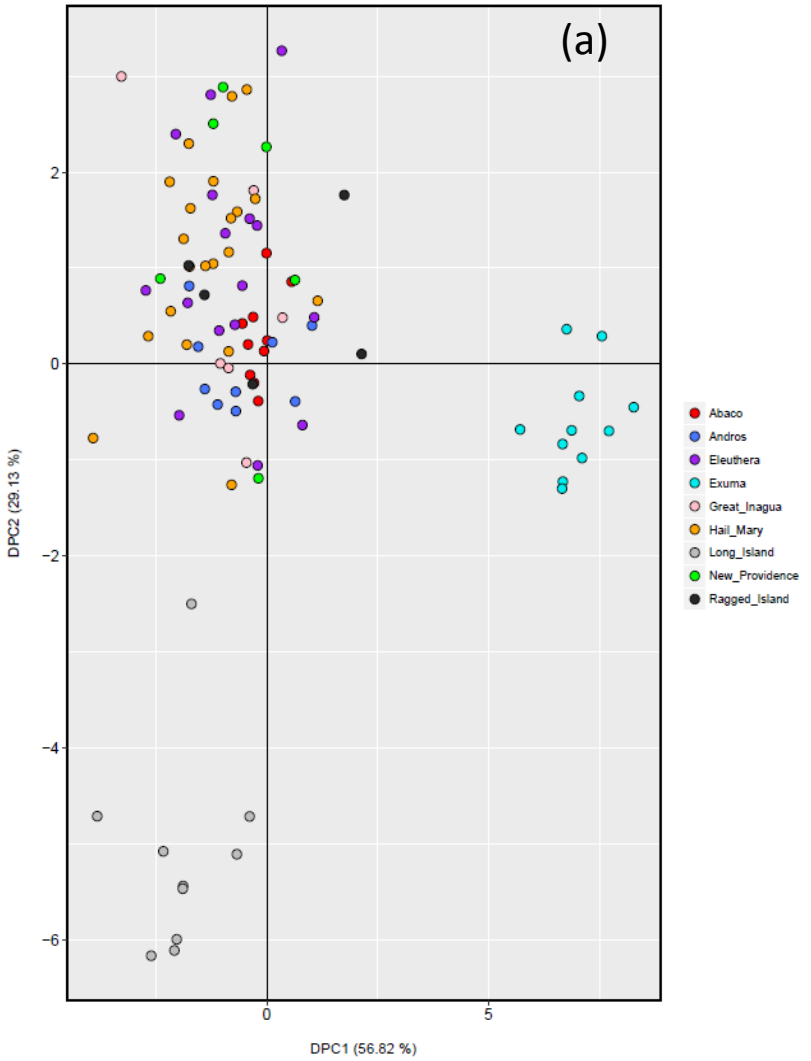
Eleuthera	**0.085**	**0.025**	--	**0.021**	**0.137**	0.004	**0.048**	**0.089**	**0.195**
Exuma	**0.087**	**0.047**	**0.021**	--	**0.164**	**0.025**	**0.058**	**0.129**	**0.248**
Great Inagua	**0.226**	**0.176**	**0.137**	**0.164**	--	**0.139**	**0.181**	**0.267**	**0.353**
Hail Mary	**0.073**	0.012	0.004	**0.025**	**0.139**	--	**0.047**	**0.111**	**0.187**
Long Island	**0.115**	**0.039**	**0.048**	**0.058**	**0.181**	**0.047**	--	**0.171**	**0.241**
New Providence	**0.200**	**0.149**	**0.089**	**0.129**	**0.267**	**0.111**	**0.171**	--	**0.296**
Ragged Island	**0.325**	**0.247**	**0.195**	**0.248**	**0.353**	**0.187**	**0.241**	**0.296**	--

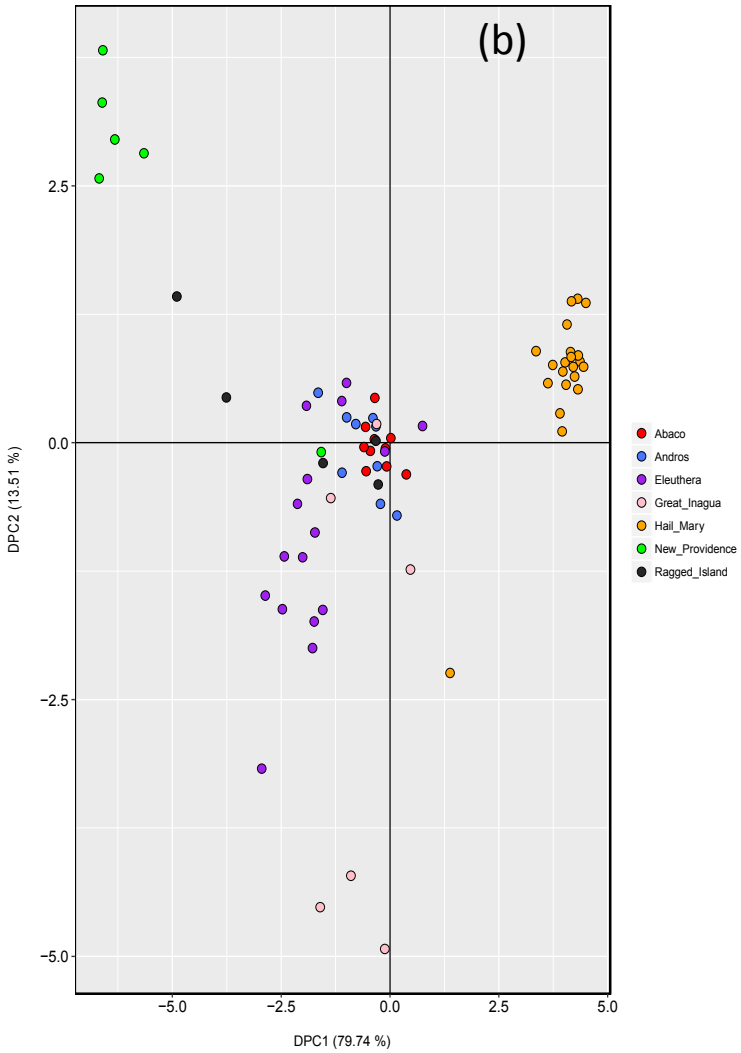
Balancing loci

	Abaco	Andros	Eleuthera	Exuma	Great Inagua	Hail Mary	Long Island	New Providence	Ragged Island
Abaco	--	-0.047	-0.037	-0.042	-0.077	-0.032	-0.052	-0.073	-0.091
Andros	-0.047	--	-0.037	-0.043	-0.072	-0.034	-0.049	-0.073	-0.083
Eleuthera	-0.037	-0.037	--	-0.033	-0.057	-0.022	-0.035	-0.057	-0.069
Exuma	-0.042	-0.043	-0.033	--	-0.064	-0.028	-0.043	-0.065	-0.078
Great Inagua	-0.077	-0.072	-0.057	-0.064	--	-0.054	-0.072	-0.099	-0.111
Hail Mary	-0.032	-0.034	-0.022	-0.028	-0.054	--	-0.033	-0.054	-0.069
Long Island	-0.052	-0.049	-0.035	-0.043	-0.072	-0.033	--	-0.075	-0.087
New Providence	-0.073	-0.073	-0.057	-0.065	-0.099	-0.054	-0.075	--	-0.108
Ragged Island	-0.091	-0.083	-0.069	-0.078	-0.111	-0.069	-0.087	-0.108	--

Hierarchical DAPC analyses (using only neutral SNPs) revealed distinct genetic clusters of Bahamian Nassau grouper (Fig. 3). The first analysis separated Long Island and Exuma from the other locations (Fig. 3a). Subsequent analyses showed differences between Hail Mary, New Providence, Great Inagua, Ragged Island (Fig. 3b-c), until finally revealing separations between Abaco, Andros and Eleuthera (Fig. 3d). However, DAPC results differed from STRUCTURE analysis, which only detected two likely genetic clusters (ΔK=2) and no clear geographic structuring (Fig. 4).

Based on AMOVA results, most genetic variability (~97 %) occurs within individuals (Table 3). However, of the three AMOVA models, the third model, which separated Exuma and Long Island versus the rest of The Bahamas, explained most of the genetic variance (p=0.055), compared with the other two models comparing the rest of The Bahamas and Exuma (p=0.111) or the rest of The Bahamas and Long Island (p=0.334; Table 3).


(a)
DPC2 (29.13 %)
DPC1 (56.82 %)
2
0
−2
−4
−6
0
5
Abaco
Andros
Eleuthera
Exuma
Great_Inagua
Hail_Mary
Long_Island
New_Providence
Ragged_Island


(b)
DPC2 (13.51 %)
DPC1 (79.74 %)
2.5
0.0
−2.5
−5.0
−5.0
−2.5
0.0
2.5
5.0
Abaco
Andros
Eleuthera
Great_Inagua
Hail_Mary
New_Providence
Ragged_Island

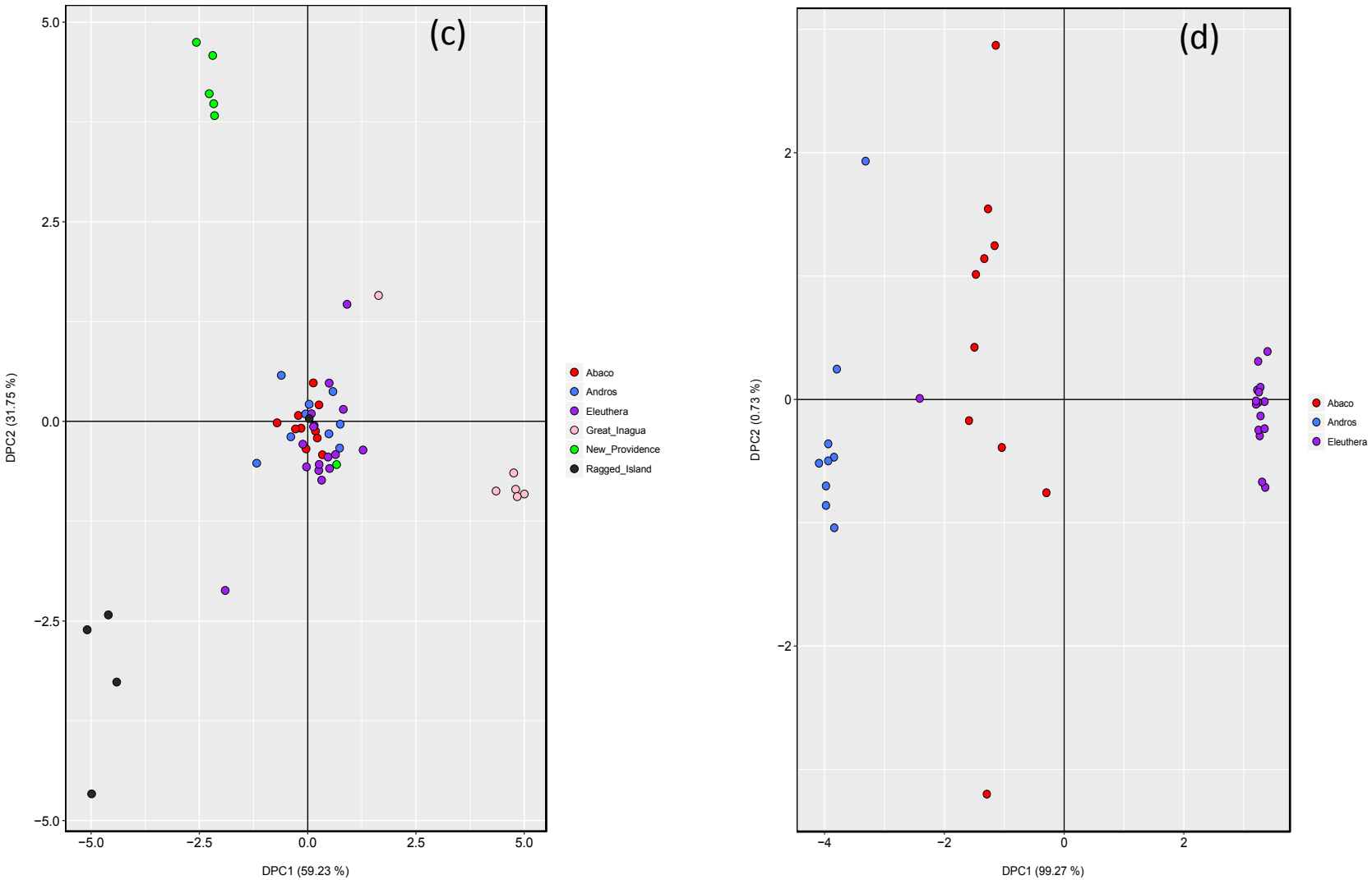


Figure 3. Hierarchical DAPC analyses of Nassau grouper depicting: a) separations of Long Island and Exuma from other

locations, b-c) differences between Hail Mary, New Providence, Great Inagua, Ragged Island, and d) separations between Abaco, Andros and Eleuthera. The per cent variation explained by each axis is presented within brackets.

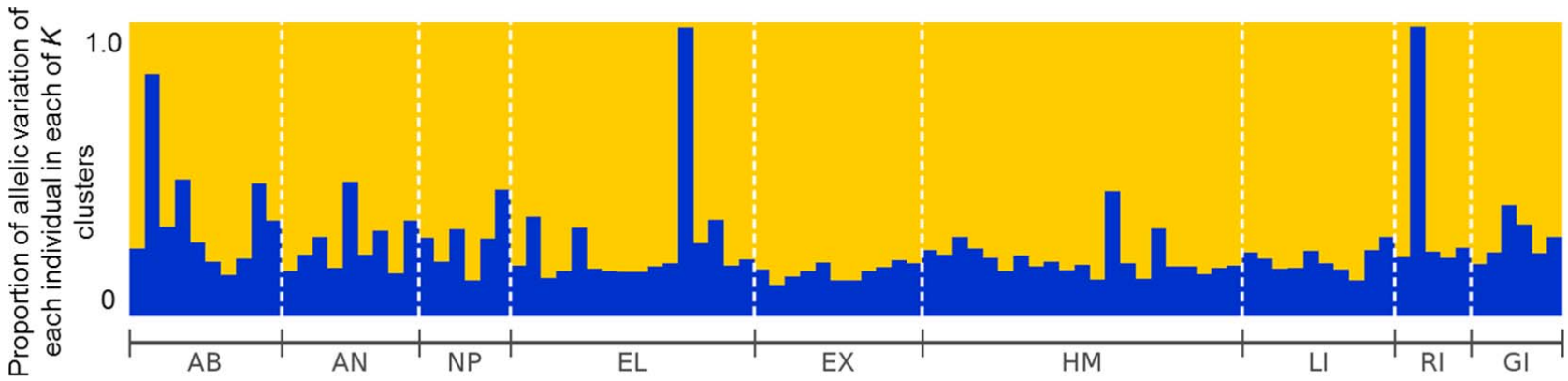


Figure 4. STRUCTURE plot depicting (*K*=2) for Nassau grouper sampled from nine locations within The Bahamas. Location codes are as follows: AB = Abaco, AN = Andros, NP = New Providence, EL = Eleuthera, EX = Exuma, HM = Hail Mary, LI = Long Island, RI = Ragged Island, and GI = Great Inagua.

Table 3. Analysis of Molecular Variance (AMOVA) for the three models tested to examine genetic variation in 94 Nassau grouper sampled from eight islands and one spawning site in The Bahamas. AMOVA 1: The Bahamas with Exuma nested, AMOVA 2: The Bahamas with Long Island nested, and AMOVA 3: The Bahamas versus Exuma versus Long Island.

AMOVA summary:								
Source of Variation	**Nested in**	**% var**	**F-stat**	**F-value**	**Std.Dev.**	**c.i.97.5 %**	**P-value**	**F'-value**
AMOVA 1								
Within Individual	--	0.972	F_it	0.028	0.002	0.032	--	--
Among Individual	Population	0.028	F_is	0.028	0.002	0.031	0.000	--
Among Population	Exuma_only	-0.002	F_sc	-0.002	0.000	-0.001	1.000	-0.002
Among Exuma_only	--	0.002	F_ct	0.002	0.000	0.003	0.111	0.003
AMOVA 2								
Within Individual	--	0.973	F_it	0.027	0.002	0.031	--	--
Among Individual	Population	0.028	F_is	0.028	0.002	0.032	0.000	--
Among Population	Long_Island_only	-0.001	F_sc	-0.001	0.000	-0.001	1.000	-0.002
Among Long_Island_only	--	0.001	F_ct	0.001	0.000	0.002	0.334	0.001
AMOVA 3								
Within Individual	--	0.972	F_it	0.028	0.002	0.032	--	--
Among Individual	Population	0.028	F_is	0.028	0.002	0.032	0.000	--
Among Population	Bah_vs_EX_vs_LI	-0.002	F_sc	-0.002	0.000	-0.001	1.000	-0.002
Among Bah_vs_EX_vs_LI	--	0.002	F_ct	0.002	0.000	0.002	0.055	0.002

Estimates of N_e based on all loci were variable, ranging from 2,216.6 (1,529.5–4,022; 95 % CI) for Long Island to infinity for five locations (Table 4). Neutral derived N_e values and confidence intervals were also all infinite (Table 4).

Table 4. N_e estimates from NeEstimator for all loci (n=10,031) and neutral loci (n=26,885) with 95 % parametric confidence intervals (CI).

Population	N	All loci N_e (95 % CI)	Neutral loci N_e (95 % CI)
Abaco	10	3,274 (1,301.9–∞)	Infinite (∞–∞)
Andros	9	2,416.9 (1,297.7–17,388.5)	Infinite (∞–∞)
Eleuthera	16	Infinite (3,179,926.3–∞)	Infinite (∞–∞)
Exuma	11	Infinite (9,978.8–∞)	Infinite (∞–∞)
Great Inagua	6	Infinite (∞–∞)	Infinite (∞–∞)
Hail Mary FSA	21	4,770 (3,431.2-7,818.8)	Infinite (∞–∞)
Long Island	10	2,216.6 (1,529.5–4,022.0)	Infinite (∞–∞)
New Providence	6	Infinite (∞–∞)	Infinite (∞–∞)
Ragged Island	5	Infinite (∞–∞)	Infinite (∞–∞)

Identifying Loci Under Selection

No outliers were found using Bayescan (Supplementary Fig. 2). However, 212 candidate loci under balancing selection (n=69 after FDR correction) and 181 under positive or diversifying selection (n=58 after FDR correction) were detected by Lositan (Supplementary Fig 3). Attempted and simulated F_{ST} values were 0.006338 and 0.004671 respectively. BLAST results generated 36 and 30 hits for loci under balancing and diversifying selection respectively, including information on predicted or known genes, proteins, and sequences associated with various biological functions (Table 5; Supplementary Tables 3–5). Multivariate DAPC analysis of neutral and positive outlier loci revealed three and four clusters respectively (Fig. 6; Supplementary Fig. 4). Conversely, DAPCs of loci under balancing selection showed no structure (Supplementary Fig. 4).

Table 5. BLAST results for putative loci under positive selection. The locus ID, coding region or distance to the nearest coding region, top species hits, number of SNPs, mean expected (H_E) and observed (H_O) heterozygosity, nucleotide diversity (π), inbreeding coefficient (F_{IS}), % query cover and identity scores are reported.

Locus ID	Coding region/distance to nearest coding region and genes	Top Species Hits	Top Species Common Names	No. SNPs	H_E	H_O	π	F_{IS}	Query Cover (%)	Identity Score (%)
1618	Damage specific DNA binding protein (ddb1)	*Labrus bergylta, Paralichthys olivaceus, Notothenia coriiceps*	ballan wrasse, olive flounder, black rockcod	1	0.38	0.40	0.41	-0.01	40	100
1735	Calcium voltage-gated channel (cacna1s)	*Notothenia coriiceps, Monopterus albus, Acanthochromis polyacanthus*	black rockcod, Asian swamp eel, spiny chromis	1	0.41	0.42	0.43	0.02	97-100	96-97
1998	retrotransposon:BEL32-LTR DR, retrotransposon:BEL32-I DR, transposon:piggyBac-N3 DR, LINE:Expander, LINE:Bridge2(Xena), LINE:Rex1 FurC, clone: 180O20	*Haplochromis chilotes, Tetraodon nigroviridis*	African cichlid	4	0.20	0.11	0.22	0.35	87	82
	retransposable elements	*Tetraodon nigroviridis*	green spotted puffer						44	95
	uncharacterized LOC107835383 (LOC107835383), ncRNA	*Poecilia formosa*	Amazon molly						48	91

3144	zinc binding - zinc finger homeobox protein 3-like, transcript variant mRNA (LOC104918143)	*Larimichthys crocea, Lates calcarifer, Notothenia coriiceps*	large yellow croaker, barramundi, black rockcod	1	0.36	0.37	0.39	0.03	100	93
4343	neuropilin-1a-like transcript variant mRNA (LOC109967076)	*Monopterus albus*	Asian swamp eel	2	0.41	0.43	0.45	0.04	100	97
	neuropilin-1a-like transcript variant mRNA (LOC109989895)	*Labrus bergylta*	ballan wrasse						100	97
	neuropilin-1a-like transcript variant mRNA (LOC104934053)	*Larimichthys crocea*	large yellow croaker						100	97
4748	protein FAM117A-like mRNA (LOC104946771)	Notothenia coriiceps	black rockcod	1	0.42	0.38	0.44	0.15	68	92
5595	collagen alpha-1(XXVIII) chain (LOC104939025), mRNA (LOC104939025, LOC109998694, LOC104951329)	*Larimichthys crocea, Labrus bergylta, Notothenia coriiceps*	large yellow croaker, ballan wrasse, black rockcod	4	0.38	0.20	0.40	0.45	54-75	88-96
6112	protein-methionine sulfoxide oxidase mical3a-like, transcript variant mRNA (LOC109961790, LOC108875448, LOC105018133)	*Monopterus albus, Lates calcarifer, Esox lucius*	Asian swamp eel, barramundi, northern pike	1	0.21	0.18	0.22	0.16	94-100	91-96

6156	Protein	*Lates calcarifer, Acanthochromis polyacanthus*	barramundi, spiny chromis	2	0.34	0.37	0.36	-0.02	100	91-93
	histone methylation protein	*Notothenia coriiceps*	black rockcod						97	93
10799	chromosome sequence corresponding to linkage group 18, complete sequence	*Dicentrarchus labrax*	European bass	2	0.22	0.17	0.23	0.09	97	89
	clusterin like 1 (clul1), transcript variant mRNA	*Larimichthys crocea*	large yellow croaker						97	88
	clusterin-like protein 1, mRNA (LOC110946200)	*Acanthochromis polyacanthus*	spiny chromis						100	82
10983	*Epinephelus coioides* x *Epinephelus lanceolatus* voucher ECEL001 microsatellite ECELEB009 sequence	*Epinephelus coides, Epinephelus lanceolatus*	orange-spotted grouper, giant grouper	3	0.36	0.30	0.38	0.19	100	92

13453	Interferon alpha 1-like gene, complete sequence; growth hormone 1 gene, complete cds; and skeletal muscle sodium channel alpha subunit-like, myosin alkali light chain-like, and microtubule-associated protein Tau-like genes	*Salmo salar*	Atlantic salmon	3	0.29	0.29	0.31	0.04	95	92
	transposase (pG-ON6-9 transposon Tc1-like)	*Oncorhynchus nerka*	sockeye salmon						95	91
	SSTN12 tn gene for putative transposase	*Oncorhynchus nerka*	sockeye salmon						95	90
	heat shock protein gene (HSP70)	*Coregonus clupeaformis*	lake whitefish						95	89
21011	semaphorin-3D-like (LOC102311894), transcript variant mRNA	*Haplochromis burtoni*	African cichlid	1	0.23	0.24	0.25	-0.01	94	91
21034	integrin alpha-M-like, mRNA (LOC108237264)	*Kryptolebias marmoratus*	mangrove rivulus	3	0.36	0.28	0.39	0.24	35	100
22628	leucine rich repeat containing G protein-coupled receptor 5 (lgr5), mRNA	*Lates calcarifer, Stegastes partitus*	barramundi, bicolor damselfish	5	0.35	0.36	0.37	0.00	100	92

25270	strain HNI chromosome 15, Hd-rR chromosome 15 sequence and complete genome assembly, strain HSOK chromosome 15	*Oryzias latipes*	Japanese rice fish	4	0.20	0.22	0.22	0.00	38	97
28915	nuclear factor 1 X-type-like, partial mRNA (LOC109644827)	*Paralichthys olivaceus*	olive flounder	3	0.35	0.24	0.38	0.27	98	95
	nuclear factor I X (nfix), transcript variant X10, mRNA	*Oreochromis niloticus*	Nile tilapia						80	100
	nuclear factor 1 X-type-like, transcript variant X10, mRNA (LOC110955692)	*Acanthochromis polyacanthus*	spiny chromis						84	98
29678	*Epinephelus fuscoguttatus* microsatellite EFJ007 sequence,	*Epinephelus fuscoguttatus*	brown marbled grouper	4	0.38	0.49	0.40	-0.19	33	100
	clone 124-1 epinecidin 1 gene, promoter region	*Epinephelus coioides*	orange-spotted grouper						33	97
30881	actin related protein 2 homolog mRNA (actr2)	*Paralichthys olivaceus, Kryptolebias marmoratus*	olive flounder, mangrove rivulus	1	0.18	0.19	0.19	-0.01	79-80	93-99
	actin related protein 2-A (LOC104930021)	*Larimichthys crocea*	large yellow croaker						79	96

31184	solute carrier family 13 (sodium-dependent citrate transporter), member 5, mRNA (slc13a5)	*Austrofundulus limnaeus*	killifish	2	0.41	0.41	0.44	0.05	79	92
36189	collagen alpha-1(XI) chain-like, transcript variant, mRNA (LOC109629802)	*Paralichthys olivaceus*	olive flounder	1	0.22	0.07	0.24	0.34	30	100
40496	ST3 beta-galactoside alpha-2,3-sialyltransferase 4 (st3gal4), transcript variant, mRNA	*Paralichthys olivaceus*	olive flounder	2	0.29	0.20	0.30	0.24	65.8	95
43923	chromosome sequence corresponding to linkage group 18, complete sequence	*Dicentrarchus labrax*	European bass	1	0.18	0.21	0.20	0.00	76	96
	strain HSOK chromosome 20	*Oryzias latipes*	Japanese rice fish						56	91
	strain HNI chromosome 20	*Oryzias latipes*	Japanese rice fish						56	91
45856	protein piccolo-like, transcript variant, mRNA (LOC108875125)	*Lates calcarifer, Larimichthys crocea, Hippocampus comes*	barramundi, large yellow croaker, tiger tail seahorse	3	0.41	0.38	0.44	0.16	54-60	94-97

49058	clone Pf_1879 ribosome-binding protein 1 (Rrbp1) mRNA, partial cds	*Perca flavescens*	yellow perch	5	0.23	0.26	0.24	-0.06	97	93
50851	kin of IRRE like 2, transcript variant, mRNA (kirrel2)	*Lates calcarifer, Cyprinodon variegatus*	barramundi, sheepshead minnow	1	0.31	0.33	0.34	0.01	66	91-97
58317	chromosome sequence corresponding to linkage group 1, top part, complete sequence	*Dicentrarchus labrax*	European bass	2	0.36	0.31	0.39	0.20	78	86
	microsatellite Luer6 sequence	*Lutjanus erythropterus*	crimson snapper						70	86
58835	succinate dehydrogenase complex assembly factor 1 (sdhaf1), mRNA	*Larimichthys crocea, Sparus aurata, Stegastes partitus*	large yellow croaker, gilt-head bream, bicolor damselfish	2	0.14	0.04	0.15	0.29	94-100	92-94
59159	common carp genome, scaffold: LG28, chromosome: 28	*Cyprinus carpio*	common carp	1	0.34	0.43	0.36	-0.17	31	100
	clone CH211-129M12 in linkage group 13, complete sequence	*Danio rerio*	zebrafish						29	100
	clone DKEYP-2B10 in linkage group 7, complete sequence	*Danio rerio*	zebrafish						29	100

59242	THUMP domain containing 2, transcript variant, mRNA (thumpd2)	*Oreochromis niloticus, Stegastes partitus, Neolamprologus brichardi*	Nile tilapia, bicolor damselfish, African cichlid	4	0.32	0.24	0.34	0.22	90-93	90-91

Gene flow

Assessments of contemporary gene flow indicate that most gene flow originates from and is retained within islands, although there is also considerable connectivity between islands throughout The Bahamas (Fig. 5). However, gene flow appears to be greater into Exuma and Eleuthera. Nassau grouper from Eleuthera, Long Island, and Andros were the main contributors to the pattern observed in Exuma. For Eleuthera, most contemporary gene flow is derived from Nassau grouper in Abaco, Andros and New Providence (Fig. 5).

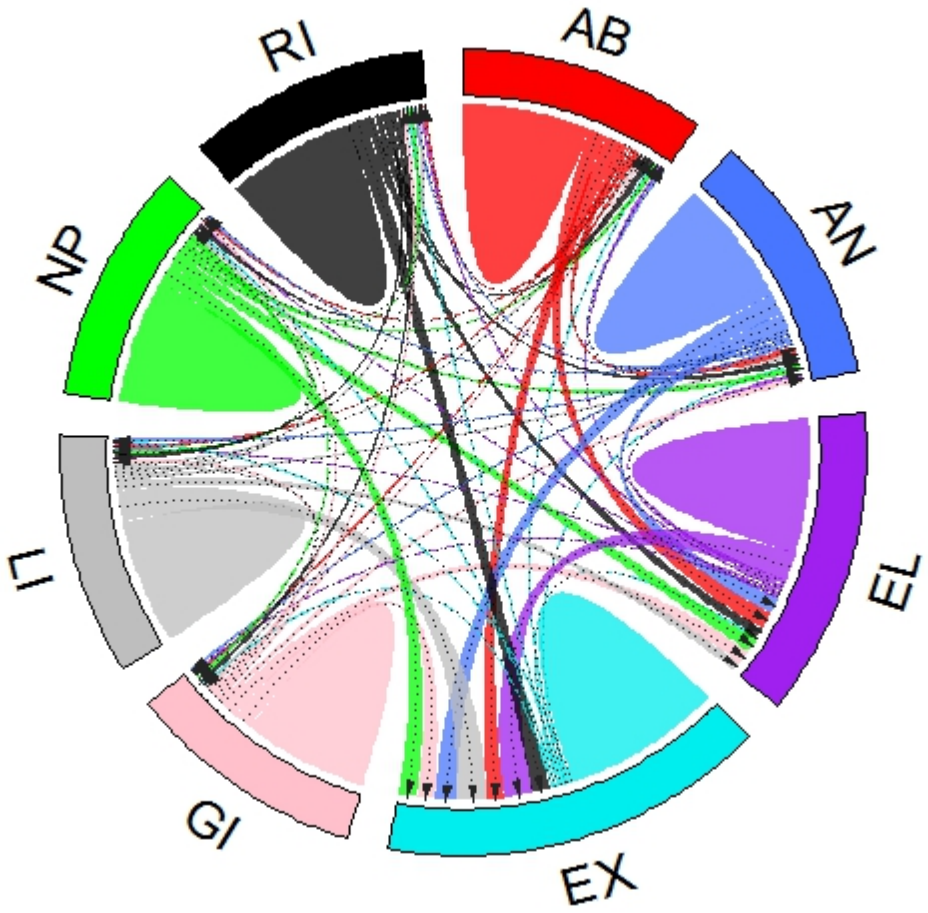


Figure 5. Contemporary gene flow diagram of Nassau grouper derived from BayesAss migration rates. Each location is represented by a different colour: Abaco (AB) = red, Andros (AN) = blue, Eleuthera (EL) = purple, Exuma (EX) = light blue, Great Inagua (GI) = pink, Long Island (LI) = grey, New Providence (NP) = green and Ragged Island (RI) = black. Humps correspond to self-recruitment (i.e.

gene flow originates from sites within the designated location (island). Arrows denote the directionality of gene flow, with arrow thickness indicating the relative contribution of gene flow.

Tagging and Telemetry

Externally tagged Nassau grouper ranged in size from 21.2–73 cm TL, with an average of 51.97 (± 14.0 SD) cm TL (Supplementary Table 6). Local fishers reported only five tag returns from the 103 Floy™ tagged individuals. Acoustically tagged fish ranged in size from 53.1–73 cm TL, averaging 63.84 (± 4.2 cm TL) and 4.5 (± 1.1 SD) kg in weight (Supplementary Table 6). Hail Mary VR2Ws recorded a total of 104,704 detections from 22 individual Nassau grouper, including 3 fish (57266, 15790, and 15791) that were tagged prior to December 2016 (Table 6). Fish 57266 was tagged in December 2015, arrived at Hail Mary on the day of the full moon (December 13th, 2016) and remained within detection range at the FSA for approximately four hours prior to its departure.

Five Nassau grouper (57267, 57276, 57280, 57284, 57285) that were acoustically tagged at the Hail Mary FSA in December 2016 were detected on two receivers in the Exuma Cays Land and Sea Park (ECLSP) a few days after the December full moon. Fish 57276 remained within detection range of Hail Mary VR2Ws for five days prior to departing for the ECLSP. Migrating Nassau grouper covered one-way distances of ~137 km (85 mi) from Hail Mary to Conch Cut (COCU) in the south and ~176 km (109 mi) to Shroud Cay (SHCA) in the north of the ECLSP during the 2016-2017 spawning season (Table 6). Three Nassau grouper that were surgically tagged in December 2016 at Hail Mary were detected on the Georgetown (GETO) receiver in Exuma during the 2016–2017 spawning season (Table 6). Two of these fish (57268 and 57278) were detected within 1–2 days after the December 13th, 2016 full moon and the other fish (57277) was detected on January 4th, 2017 (Table 6). Migrating Nassau grouper travelled ~15.7 km (9.8 mi) from Hail Mary to GETO.

No Nassau grouper were detected on the Poseidon Point (POPO) receiver on the eastern side of Long Island. However, one individual (57263) was detected

on the Newton Cay (NECA) VR2W for 27 consecutive days beginning November 28th, 2015 and then again for another four days around the January 2016 full moon (Table 6).

Table 6. Details of acoustic telemetry data including transmitter identification (ID), tag date, detection information (i.e. dates, times, number of detections and receiver locations) for n=23 Nassau grouper detected on receivers in the Exuma Sound. Receiver codes are as follows: SHCA = Shroud Cay, COCU = Conch Cut, GETO = Georgetown, HMN = Hail Mary North, HME = Hail Mary East, HMC = Hail Mary Central, HMW = Hail Mary West, HMS = Hail Mary South, NOPO = North Point, NECA = Newton's Cay, POPO = Poseidon's Point.

Transmitter ID	Tag Date	Initial Detection Date	Dec. 2016 Arrival Date	Dec. 2016 Arrival Time	Dec. 2016 Departure Date	Dec. 2016 Departure Time	Jan. 2017 Arrival Date	Jan. 2017 Arrival Time	Jan. 2017 Departure Date	Jan. 2017 Departure Time	Final Detection Date	No. of Detections	Initial-Final Station Location
57263	10-Sep-15	28-Nov-15	—	—	—	—	22-Jan-16	5:58	—	—	26-Jan-16	1566	NECA-NECA
57266	26-Dec-15	13-Dec-16	13-Dec-16	4:46	13-Dec-16	8:51	—	—	—	—	13-Dec-16	16	HMN-HME
15790	24-Jan-16	25-Jan-16	25-Jan-16	16:38	26-Jan-16	19:10	—	—	—	—	26-Jan-16	325	HMC-GETO
15791	24-Jan-16	25-Jan-16	25-Jan-16	19:23	28-Sep-16	16:52	—	—	—	—	28-Sep-16	90322	HMN-HMN
57268	13-Dec-16	13-Dec-16	13-Dec-16	17:15	14-Dec-16	21:14	—	—	—	—	14-Dec-16	635	HMC-GETO
57269	13-Dec-16	13-Dec-16	13-Dec-16	18:25	14-Dec-16	12:52	7-Jan-17	14:30	11-Jan-17	8:04	11-Jan-17	857	HMN-HMS
57270	13-Dec-16	13-Dec-16	13-Dec-16	18:26	14-Dec-16	11:40	—	—	—	—	14-Dec-16	638	HMN-HMS
57271	13-Dec-16	13-Dec-16	13-Dec-16	21:14	14-Dec-16	11:51	6-Jan-17	11:10	6-Jan-17	14:52	6-Jan-17	771	HMC-HME
57267	14-Dec-16	14-Dec-16	14-Dec-16	15:17	14-Dec-16	22:57	—	—	—	—	20-Dec-16	268	HMC-COCU
57272	14-Dec-16	14-Dec-16	14-Dec-16	18:35	15-Dec-16	7:32	—	—	—	—	15-Dec-16	345	HMC-HMS
57273	14-Dec-16	14-Dec-16	14-Dec-16	15:54	15-Dec-16	9:00	—	—	—	—	15-Dec-16	678	HMN-HMS
57274	14-Dec-16	14-Dec-16	14-Dec-16	17:18	15-Dec-16	10:27	5-Jan-17	14:54	5-Jan-17	17:32	5-Jan-17	460	HMN-HMS
57275	14-Dec-16	14-Dec-16	14-Dec-16	17:37	15-Dec-16	4:50	—	—	—	—	15-Dec-16	271	HMC-HMS
57276	14-Dec-16	14-Dec-16	14-Dec-16	18:37	19-Dec-16	13:23	—	—	—	—	28-Dec-16	4150	HMC-COCU
57277	14-Dec-16	14-Dec-16	14-Dec-16	21:49	15-Dec-16	11:38	4-Jan-17	17:35	5-Jan-17	16:04	5-Jan-17	560	HMC-HMS
57278	14-Dec-16	14-Dec-16	14-Dec-16	20:25	16-Dec-16	12:07	—	—	—	—	16-Dec-16	563	HMC-GETO
57279	14-Dec-16	14-Dec-16	14-Dec-16	20:39	15-Dec-16	11:47	—	—	—	—	15-Dec-16	313	HMN-HMS
57280	14-Dec-16	14-Dec-16	14-Dec-16	17:24	15-Dec-16	11:30	—	—	—	—	20-Dec-16	529	HMC-COCU
57281	14-Dec-16	14-Dec-16	14-Dec-16	18:52	15-Dec-16	11:33	5-Jan-17	14:50	6-Jan-17	14:39	6-Jan-17	624	HMN-HME
57282	14-Dec-16	14-Dec-16	14-Dec-16	21:50	15-Dec-16	9:33	5-Jan-17	14:57	5-Jan-17	16:22	5-Jan-17	451	HMS-HME

57283	14-Dec-16	14-Dec-16	14-Dec-16	21:21	15-Dec-16	19:00	6-Jan-17	20:35	12-Jan-17	11:54	12-Jan-17	1175	HMC-HMS
57284	14-Dec-16	14-Dec-16	14-Dec-16	21:50	15-Dec-16	11:46	—	—	—	—	22-Feb-17	334	HMC-SHCA
57285	14-Dec-16	14-Dec-16	14-Dec-16	15:13	15-Dec-16	18:07	—	—	—	—	22-Dec-16	419	HMC-COCU

Discussion

For this research, RAD-seq was used to discover SNPs for Nassau grouper, enabling assessments of genomic diversity, population divergence, N_e and gene flow within The Bahamas. Additionally, the role of selection on genetic population structure of Nassau grouper was investigated for the first time. Analyses of high-resolution SNP markers (including candidate loci under divergent selection) coupled with acoustic telemetry helped to resolve patterns of spatial and genetic connectivity of Bahamian Nassau grouper, and to determine the origins of individuals migrating to the Hail Mary FSA.

Genetic Diversity, Differentiation and Structure

Global estimates of genetic differentiation using the full dataset (all loci) and neutral loci (Global F_{ST}=0.0003, p=0.325 and F_{ST}=0.0003, p=0.325) were lower than that reported by Jackson et al. (2014) using SNPs (F_{ST}=0.002, p=0.014) and microsatellite markers (F_{ST}=0.002, p=0.004) respectively. This finding however, is not surprising given that Nassau grouper in the Jackson et al. (2014) study were sampled over an extensive geographical area, encompassing The Bahamas and parts of the Caribbean. Measures of gene diversity reported here (H_E=0.340–0.373, π=0.337–0.372) are greater than SNP-based nucleotide diversity values for several other marine species, e.g., small yellow croaker (*Larimichthys polyactis*) H_E=0.216, π=0.00105 (Zhang et al. 2016), chinook salmon (*O. tshawytscha*) H_E=0.232–0.261 (Larson et al. 2014), and spotted sea bass (*Lateolabrax maculatus*) H_E=0.281–0.30, π=0.002–0.003 (Wang et. al. 2016). Possible explanations for the disparity between microsatellites versus SNP data include the bi-allelic nature of SNPs and their slower mutation rates (Seeb et al. 2011). However, genome-wide assessments of diversity and differentiation with large numbers of SNPs have been shown to yield more accurate estimations of patterns of genetic variation (Helyar et al. 2011; Fischer et al. 2017). While there are differences in gene diversity values for Nassau grouper reported using microsatellites and SNP markers in two studies (Jackson et al. 2014; Bernard et al. 2016); the RAD-seq data presented in this study concur with microsatellite data by Sherman et al. (2017), with both techniques demonstrating similar levels of genetic diversity in Bahamian Nassau grouper.

STRUCTURE analysis of genotyped Nassau grouper based on 10,031 SNPs revealed no geographic genetic population structure, which is also in accordance with microsatellite-based analysis of Bahamian Nassau grouper (Sherman et al. 2017). However, using neutral loci, hierarchical multivariate DAPC analyses enabled detection of distinct genetic clusters. The first DAPC analysis yielded three groups, explained by ~57 % and 29 % of the variation; while the second DAPC provided further separation of fish from the Hail Mary FSA, with the axes accounting for ~80 % and 14 % of the variation respectively. Discrepancies between DAPC and STRUCTURE analyses have been documented in previous studies (e.g., Jombart et al. 2010; Overgaard Therkildsen et al. 2013; Benestan et al. 2015; Vergara et al. 2015). These inconsistencies have been attributed to differences in the underlying assumptions and models used for STRUCTURE versus DAPC analysis, the latter being a multivariate approach that attempts to simultaneously maximise variability between populations, while minimising variation within populations. As a result, DAPC is often cited as an improved method for revealing fine-scale patterns of population structure in comparison to Bayesian-based STRUCTURE, which appears to have limited power to delineate structure when population differentiation estimates are low (Jombart et al. 2010; Kalinowski 2010). Therefore, the observed genomic divergences of Exuma and Long Island from the rest of The Bahamas, despite exhibiting similar levels of heterozygosity, infer that subtle differences in gene flow may be occurring among Nassau grouper from these islands.

In the present study, SNP-based estimates of contemporary N_e for Abaco, Andros, Hail Mary and Long Island were within range of N_e values produced by *VarEff* analysis for The Bahamas (Sherman et al. 2017). However, with the larger SNP dataset, several instances of infinite N_e were found, including fish from Eleuthera, Exuma, Great Inagua, New Providence and Ragged Island. These results are either indicative of truly large effective population sizes or an artefact of sampling (Larson et al. 2014) and may have been influenced by linkage disequilibrium and/or Wahlund effects (Sherman et al. 2017).

Identifying Loci Under Selection

Examination of loci under selection has been employed to explain genetic population structuring in marine species (Hohenlohe et al. 2010; Overgaard Therkildsen et al. 2013; Benestan et al. 2015), which is fundamental for understanding adaptive responses and the associated linkages between genetic diversity. Therefore, these data are useful from both an evolutionary and conservation management perspective (Funk et al. 2012). To this end, we performed DAPC analyses of both positive and balancing loci to visualise whether patterns of genetic structure could be attributed to outlier loci.

Outlier SNPs under putative divergent selection and balancing loci were connected to a spectrum of genes, proteins, and/or sequences associated with important biological functions (Table 5; Supplementary Tables 3–5). Of particular interest were multiple hits related to heat shock proteins (HSPs) including Hsp40, Hsp70 and dnajb5, which are activated as an immune/stress response to environmental, chemical or physical stimuli (Li and Srivastava 2004; Javid et al. 2007). Mean monthly water temperatures in The Bahamas range from 24 °C in the winter to 29 °C in the summer, but rarely drop below 26 °C in the southern Bahamas during winter months (https://www.seatemperature.org/central-america/bahamas/nassau.htm). However, elevated water temperatures in nursery habitats (exposed to tidal fluctuations) and on the Bahama Banks are common during summer months, where maximum temperatures can exceed 40 °C (Murchie et al. 2011; Shultz et al. 2014). In the present study, there were no clear geographic patterns related to gene diversity for outlier loci linked to HSPs (Supplementary Fig. 4; Supplementary Tables 3–5). Locus 36230 exhibited comparable levels of nucleotide diversity throughout The Bahamas. Conversely, nucleotide diversity for positive and balancing loci (13453 and 51124) was lower in Eleuthera (central Bahamas) and Great Inagua (southern Bahamas) respectively, compared to the other islands and Hail Mary. HPSs are ubiquitous within and among species (Li and Srivastava 2004), so the prevalence of these genes in loci under balancing selection seems logical.

Our results showed similarities in the numbers and spatial patterns of genetic clusters with neutral loci and all loci, but not with positive loci (Fig. 3; Supplementary Fig. 4). Pairwise F_{ST} with neutral loci showed no significant F_{ST}

values, whereas, pairwise analysis of positive loci showed virtually all significant differences in F_{ST} (Table 2), suggesting that selection may also be influencing population structure of Nassau grouper. Yet, in contrast to other research (e.g., Hess et al. 2013; Benestan et al. 2015; Xu et al. 2017), loci under potential diverging selection were unable to explain the spatial patterns observed with all loci and neutral loci in this study. For example, using SNP data obtained from RAD-seq, Hess et al. (2013) were able to link adaptive loci with environmental conditions that helped to explain demographic population structure in Pacific lamprey (*Entosphenus tridentatus*). By examining patterns of gene flow, dispersal and putative loci under adaptive selection, Moore et al. (2017) found that Arctic char (*Salvelinus alpinus*) altered their migratory patterns to reduce exposure to unfavourable environmental conditions outside of the reproductive season. Nassau grouper migrate during winter months, when water temperatures are cooler (Colin 1992). Any extremes in water temperature are likely to be experienced by juvenile and subadult fish transitioning from nursery habitats to reefs (Eggleston et al. 1998). However, long distance migrations are likely to be more energetically costly, and thus can explain the presence of outlier loci associated with osmoregulation, metabolic processes and muscle function (Supplementary Table 3).

There are, however, limitations with the different methods used for detecting loci under selection and even moderate gene flow may mask signals of divergence (Davey et al. 2011; Narum and Hess 2011). However, given the potential shortfalls associated with outlier loci testing, alternative methods (Excoffier et al. 2009; Moore et al. 2017) should be employed for cross-validation.

Gene Flow

An important goal in conservation genetics is to maximize gene flow, N_e and genetic diversity to maintain healthy and viable populations for species of interest (Frankham et al. 2014; Hemmer-Hansen et a. 2014; Miller et al. 2016). We investigated the directionality of gene flow for Nassau grouper within The Bahamas. Our analysis indicates considerable genetic exchange throughout The Bahamas, with most gene flow originating within islands. The patterns of connectivity observed are congruent with recent microsatellite analysis of

Bahamian Nassau grouper, which showed similar levels of genetic diversity despite both historic and contemporary natural and anthropogenic events, suggesting that gene flow may be an important driver of contemporary genetic structure (Sherman et al. 2017). However, results from the present study, have also shown that genetic exchange appears to be more substantial flowing into the Exumas and Eleuthera, with reduced gene flow emanating out from these islands (Fig. 5).

In the absence of overt physical barriers to gene flow, probable explanations for the observed patterns of genetic connectivity include, 1) varied migration patterns and adult dispersal capabilities (Bolden 2000; Dahlgren et al. 2016a); 2) intraspecific differences in larval survivorship, dispersal and recruitment (Eggleston 1995; Kough and Paris 2015); 3) low densities of Nassau grouper (0.13−0.25 fish/100 m^2) outside the ECLSP (Dahlgren et al. 2016b; Sherman et al. In Review); and 4) divergent selective pressures between islands (e.g., Delmore et al. 2015; Moore et al. 2017). As a transient spawner (Domeier 2012), most of the reproductive effort for Nassau grouper is limited to a relatively narrow temporal window, which is further influenced by environmental and oceanographic processes (e.g., water temperature, currents and the lunar cycle) (Colin 1992; Colin 2012). Through biophysical modelling, Kough and Paris (2015) demonstrated that infrequent spawners often exhibit reduced larval connectivity networks. Therefore, heavy exploitation of Nassau grouper outside the MPA coupled with reduced larval connectivity networks may potentially limit the relative contribution of gene flow throughout parts of The Bahamas. While residents of the ECLSP experience negligible fishing pressure compared to the rest of The Bahamas (Dahlgren et al. 2016b), telemetry data in the present study, as well as previous research (Bolden 2000; Dahlgren et al. 2016a), support connectivity through adult migrations for spawning, which are variable in space and time.

Approximately 37 % of Nassau grouper from the Exuma Cays were sampled within the ECLSP (Supplementary Table 6). Gene flow analysis suggests that this no-take MPA may be acting as a sink for contemporary Nassau grouper populations in The Bahamas. Previous studies have also shown reduced larval recruitment into the ELCSP for two other commercially valuable fishery species – Caribbean spiny lobster (*Panulirus argus*) and queen conch (*Lobatus gigas*) (Stoner et al. 2012; Lipcius et al. 2013), implying that the

MPA functions as a sink for these species. Contemporary gene flow patterns and mostly significant pairwise F_{ST} values for putative loci under positive selection corroborate DAPC and AMOVA analyses and help to explain the observed fine-scale intraspecific population structure (Figs. 3–5).

Genetic connectivity in marine species is often complicated by geographical separation, migration, larval dispersal, life history characteristics (e.g., age at maturity, ontogeny, reproductive strategies), and oceanographic conditions (Rivera et al. 2011; Hemmer-Hansen et al. 2014; Selkoe et al. 2016). Additional research is required to clarify patterns of gene flow and provide additional insights into the mechanisms influencing source-sink dynamics for Nassau grouper throughout The Bahamas. Such data will have important implications for designing MPA networks, including the protection of specific FSAs and recommending fishery quotas.

Tagging and Telemetry

Round-trip migration distances covered by Nassau grouper in The Bahamas can exceed 300 km (Dahlgren et al. 2016a), thus surpassing distances reported for fish in in the Cayman Islands and Belize (e.g., Whaylen et al. 2004; Semmens et al. 2007; Starr et al. 2007). However, the migratory patterns displayed by Nassau grouper in this study are congruent with previous research in The Bahamas in terms of routes and distances (~15.7 to 176 km) travelled (Bolden 2000; Dahlgren et al. 2016a; Stump et al. 2017). Adult Nassau grouper migrate along the shelf edge in groups to and from FSAs within 1–2 weeks of the full moon (Dahlgren et al. 2016a; Stump et al. 2017). Moreover, Dahlgren et al. (2016a) demonstrated that some fish from the ECLSP utilise FSAs around Long Island and highlighted temporal differences in migratory patterns, which coincided with the timing of the full moon. In the present study, of all 44 acoustically tagged fish, 22 % were detected by receivers travelling from Hail Mary into the Exumas (Figs. 1–2), and 14 % entered the ECLSP, a no-take marine protected area (MPA). Acoustic telemetry revealed the ECLSP as the likely origin of five Nassau grouper, representing 5 of 19 individuals (26 %) tagged in December 2016. These results not only corroborate patterns of long distance adult dispersal during the winter spawning season, but also highlight the relative contribution of the ECLSP to FSAs around Long Island.

Conclusion

Here, we aimed to determine whether a genomic approach, in conjunction with acoustic telemetry, could resolve intraspecific population structure and provide additional insight into the demographics of Nassau grouper in The Bahamas. RAD-seq analysis using more than 13,000 SNPs yielded new genomic data for the species. Key findings from this research show that genomic patterns of diversity in Nassau grouper exhibit a high degree of uniformity throughout The Bahamas. This pattern was reflective of STRUCTURE analysis and pairwise F_{ST} data from all loci, showing limited differentiation within the archipelago. However, DAPC analyses of neutral loci in Nassau grouper revealed hierarchical genetic clustering that was not detected via STRUCTURE analysis.

Patterns of cryptic divergence were further supported by AMOVAs and gene flow analysis, showing differences in the relative contribution of genes in parts of The Bahamas. Gene flow analysis, however, also showed that the ECLSP appears to function as a sink for Nassau grouper. This was further supported by acoustic telemetry data, which was used to confirm migratory corridors and track northwest movements of Nassau grouper from an active FSA into the Exumas and into the ECLSP. Long-distance movement patterns displayed by acoustically tagged fish provide additional evidence for a mechanism of genetic exchange, which is likely to be ubiquitous throughout The Bahamas. Collectively, these results demonstrate the critical importance of understanding connectivity for well-designed MPAs and MPA networks, as they can be valuable tools for species conservation.

Through RAD-seq, new information on putative loci associated with divergent and balancing selection in Nassau grouper was detected. It appears that selection may also be an important driver in shaping the contemporary genetic population structure of Nassau grouper as evidenced by mostly significant pairwise F_{ST} values of positive loci and warrants further exploration to identify the processes influencing selection. These findings may have implications for long-term survival in light of future anthropogenic disturbances (e.g. forecasted increases to water temperatures, which may impact the timing of migrations). Moreover, the use of SNP data will facilitate regional comparisons of genetic diversity, differentiation and structure once such data

become available from other countries where Nassau grouper populations still exist. In the future, Nassau grouper SNP data may have applications beyond demographic inferences, such as examining the extent of illegal unreported and unregulated (IUU) fishing for fisheries management (e.g., Martinsohn and Ogden 2009).

Overall, data from this study build upon previous Nassau grouper research in The Bahamas. Our research demonstrates the power of RAD-seq to reveal complex spatial patterns and provide novel insight into population dynamics of an endangered marine species. Furthermore, the application of both *in situ* methodologies (i.e. acoustic telemetry) and molecular research highlight the benefits and importance of integrative approaches for advancing understanding the dynamics of aggregating species.

Acknowledgements

Established ethical procedures reviewed by Shedd Aquarium's internal and external research committee were adhered to during animal handling. Fin clips were obtained with permission from The Bahamas Department of Marine Resources under scientific research permit (MAMR/FIS/17 – Sherman) to K. Sherman and follow UK Home Office guidelines. Fieldwork and telemetry research were completed under permits issued to K. Sherman (MAMR/FIS/17 – Sherman) and K. Stump (MAMR/FIS/17 – Stump). We are grateful to research volunteers from the Shedd Aquarium, Bahamas National Trust, Atlantis, Cape Eleuthera Institute, Bahamas Reef Environment Educational Foundation, The Nature Conservancy, University of Miami, T. Kamerman, S. Buhler, D. Rottman, D. Delaney, N. Truelove, B. Crown and R. Fluck who assisted with fieldwork between December 2014 – May 2017 and to Keith Pamper and the crew of the *R/V Coral Reef II* for logistical field support.

Fieldwork was funded by Shedd Aquarium. RAD-sequencing was funded by the Save Our Seas Foundation (small grant No. 365) and Lyford Cay Foundation via the Shirley Oakes Butler Charitable Trust scholarship to K. Sherman. Finally, we would like to thank the Exeter Sequencing Service and Computational core facilities team at the University of Exeter.

Data Accessibility

RAD-seq data will be made available upon publication.

Author Contributions

KDS conducted fieldwork, performed genomic DNA extractions and quality testing, prepared samples for RAD-tag library development, analysed the data and wrote the manuscript. KM developed RAD-tag libraries and completed sequencing. JRP assisted with bioinformatics and contributed to the manuscript. RAK provided laboratory support and advice for data analysis. CPD, KS and LRK were involved with fieldwork and commented on the manuscript. JRS and CRT contributed to the scientific discussions and helped to improve the manuscript. The final version was approved by all authors. We have no conflicting interests to declare.

References

Abelson A, Obolski U, Regoniel P, Hadany L (2016) Restocking herbivorous fish populations as a social-ecological restoration tool in coral reefs. Frontiers in Marine Science 3: 10.3389/fmars.2016.00138

Allendorf FW, Hohenlohe PA, Luikart G (2010) Genomics and the future of conservation genetics. Nature Reviews Genetics 11:697−709

Andrews KR, Good JM, Miller MR, Luikart G, Hohenlohe PA (2016) Harnessing the power of RADseq for ecological and evolutionary genomics. Nature Reviews Genetics 17: 81−92

Andrews S *(*2010*)* FastQC: a quality control tool for high throughput sequence data. Available at: http://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed 4 April 2017).

Antao T, Lopes A, Lopes RJ, Beja-Pereira A, Luikart G (2008) LOSITAN: A workbench to detect molecular adaptation based on a F_{st} outlier method. BMC Bioinformatics 9:323

Archer SK, Heppell SA, Semmens BX, Pattengill-Semmens CV, Bush PG, McCoy CM, Johnson BC (2012) Patterns of color phase indicate spawn timing at a Nassau grouper *Epinephelus striatus* spawning aggregation. Current Zoology 58:73−83

Aswani S, Mumby PJ, Baker AC, Christie P, McCook LJ, Steneck RS, Richmond RH (2015) Scientific frontiers in the management of coral reefs. Frontiers in Marine Science 2:10.3389/fmars.2015.00050

Austin M (2007) Species distribution models and ecological theory: A critical assessment and some possible new approaches. Ecological Modelling 200:1−19

Baird NA, Etter PD, Atwood TS Currey MC, Shiver AL, Lewis ZA, Selker EU, Cresko WA, Johnson EA (2008) Rapid SNP discovery and genetic mapping using sequenced RAD markers. PLoS One 3:e3376

Beaumont MA, Nichols RA (1996) Evaluating loci for use in the genetic analysis of population structure. Proceedings of the Royal Society of London. Series B: Biological Sciences 263:1619−1626

Benestan L, Gosselin T, Perrier C, Sainte-Marie B, Rochette R, Bernatchez L (2015) RAD genotyping reveals fine-scale genetic structuring and provides powerful population assignment in a widely distributed marine species, the American lobster (*Homarus americanus*). Molecular Ecology 24:3299−3315

Bernard AM, Feldheim KA, Nemeth R, Kadison E, Blondeau J, Semmens BX, Shivhi MS (2016) The ups and downs of coral reef fishes: the genetic characteristics of a formerly severely overfished but currently recovering Nassau grouper fish spawning aggregation. Coral Reefs 35:273−284

Bolden SK (2000) Long-distance movement of a Nassau grouper (*Epinephelus striatus*) to a spawning aggregation in the central Bahamas. Fishery Bulletin 98: 642−645

Bozec YM, O'Farell S, Bruggemann JH, Luckurst BE, Mumby PJ (2016) Tradeoffs between fisheries harvest and the resilience of coral reefs. Proceedings of the National Academy of Sciences USA 113:4536−4541, doi: 10.1073/pnas.1601529113

Calosi P, De Wit P, Thor P, Dupont S (2016) Will life find a way? Evolution of marine species under global change. Evolutionary Applications 9:1035−1042

Carpenter KE, Claro R, Cowan J, Sedberry G, Zapp-Sluis M (2015) *Epinephelus striatus*. The IUCN Red List of Threatened Species 2015: e.T7862A70324790. Downloaded on 19 March 2018

Catchen JM, Amores A, Hohenlohe P, Cresko W, Postlewait JH (2011) Stacks: building and genotyping loci de novo from short-read sequences. G3: Genes, Genomes, Genetics 1:171−182

Catchen J, Hohenlohe PA, Bassham S, Amores A (2013) Stacks: an analysis tool set for population genomics. Molecular Ecology 22:3124−3140

Chhatre VE, Emerson KJ (2017) StrAuto: automation and parallelization of STRUCTURE analysis. BMC Bioinformatics 18:192, doi: 10.1186/s12859-017-1593-0

Cheung, WWL, Sadovy Y, Braynen MT, Gittens LG (2013) Are the last remaining Nassau grouper *Epinephelus striatus* fisheries sustainable? Status quo in the Bahamas. Endangered Species Research 20:27−39

Cheung WWL, Sarmiento JL, Dunne J, Frölicher TL, Lam VWL, Deng Palomares ML, Watson R, Pauly D (2012) Shrinking of fishes exacerbates impacts of global ocean changes on marine ecosystems. Nature Climate Change 3:254−258

Coleman FC, Koenig CC, Huntsman GR, Musick JA, Eklund AM, McGovern JC, Sedberry GR, Chapman RW, Grimes CB (2000) Long-lived reef fishes: the grouper-snapper complex. Fisheries 25:14−21

Colin PL (1992) Reproduction of the Nassau grouper, *Epinephelus striatus*, (Pisces: Serranidae) and its relationship to environmental conditions. Environmental Biology of Fishes 34:357–377

Colin (1996) Longevity of some coral reef fish spawning aggregations Copeia 1996:189−192

Colin PL (2012) Aggregation Spawning: Biological aspects of the early life history. In: Sadovy de Mitcheson Y, Colin PL (eds) Reef fish spawning aggregations: biology, research and management. Springer, New York, NY, p 191−224

Crozier LG, Hutchings JA, (2014) Plastic and evolutionary responses to climate change in fish. Evolutionary Applications 7:68−87

Dahlgren CP, Eggelston DB (2001) Spatio-temporal variability in abundance, size and microhabitat associations of early juvenile Nassau grouper *Epinephelus striatus* in an off-reef nursery system. Marine Ecology Progress Series 217:145–156

Dahlgren CP, Buch K, Rechisky E, Hixon MA (2016a) Multiyear tracking of Nassau grouper spawning migrations. Marine and Coastal Fisheries 8:522−535

Dahlgren C, Sherman K, Lang J, Kramer PR, Marks K (2016b) Bahamas coral reef report card, Vol. 1: 2011−2013. Nassau, Bahamas, http://www.agrra.org/wp-content/uploads/2016/05/Bahamas-2016-Coral-Reef-Report-Card.pdf

Dann TH, Habicht C, Baker TT, Seeb JE, Fraser D (2013) Exploiting genetic diversity to balance conservation and harvest of migratory salmon. Canadian Journal of Fisheries and Aquatic Sciences 70:785−793

Davey JW, Hohenlohe PA, Etter PD, Boone JQ, Catchen JM, Blaxter ML (2011) Genome-wide genetic marker discovery and genotyping using next-generation sequencing. Nature Reviews Genetics 12:499−510

Delmore KE, Hübner S, Kane NC, Schuster R, Andrew RL, Câmara F, Guigó R, Irwin DE (2015) Genomic analysis of a migratory divide reveals candidate genes for migration and implicates selective sweeps in generating islands of differentiation. Molecular Ecology 8:1873−1888

Do C, Waples RS, Peel D, Macbeth GM, Tillet BJ, Ovenden JR (2014) NeEstimator V2: re-implementation of software for the estimation of contemporary effective population size (N_e) from genetic data. Molecular Ecology Resources 14:209−214

Domeier ML (2012) Revisiting Spawning Aggregations: Definitions and Challenges. In: Sadovy de Mitcheson Y, Colin PL (eds). Reef Fish Spawning Aggregations: Biology, Research and Management. Vol. 35. Springer, p. 1−20

Earl DA, vonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conservation Genetic Resources 4:359–361, doi: 10.1007/s12686-011-9548-7

Egerton JP, Johnson AF, Le Vay L, McCoy CM, Semmens BX, Heppell SA, Turner JR (2017) Hydroacoustics for the discovery and quantification of Nassau grouper (*Epinephelus striatus*) spawning aggregations. Coral Reefs 36:589−600

Eggleston DB (1995) Recruitment in Nassau grouper *Epinephelus striatus*: post-settlement abundance, microhabitat features, and ontogenetic habitat shifts. Marine Ecology Progress Series 124: 9−22

Eggleston DB, Grover JJ, Lipcius RN (1998) Ontogenetic diet shifts in Nassau grouper: trophic linkages and predatory impact. Bulletin of Marine Science 63:111−126

Eizaguirre C, Baltazar-Soares M (2014) Evolutionary conservation—evaluating the adaptive potential of species. Evolutionary Applications 7:963−967

Etter PD, Bassham S, Hohenlohe PA, Johnson EA, Cresko WA (2011) SNP discovery for evolutionary genetics using RAD sequencing. In: Molecular Methods for Evolutionary Genetics, Methods in Molecular Biology. Orgogozo V and Rockman MV (eds). 772:157−178. Heidelberg: Springer. doi:10.1007/978-1-61779-228-1_9

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software structure: a simulation study. Molecular Ecology 14:2611–2620

Excoffier L, Hofer T, Foll M (2009) Detecting loci under selection in a hierarchically structured population. Heredity 103:285−298

Fischer MC, Rellstab C, Leuzinger M, Roumet M, Gugerli F, Shimizu KK, Holderegger R, Widmer A (2017) Estimating genomic diversity and population differentiation – an empirical comparison of microsatellite and SNP variation in *Arabidopsis halleri*. BMC Genomics 18:69, doi:10.1186/s12864-016-3459-7

Flanagan SP, Forester BR, Latch EK, Aitken SN, Hoban S. Guidelines for planning genomic assessment and monitoring of locally adaptive variation to inform species conservation. (2017) Evolutionary Applications 00:1–18

Foll M, Gaggiotti O (2008) A genome-scan method to identify selected loci appropriate for both dominant and codominant markers: a bayesian perspective. Genetics 180:977–993

Francis RM (2017) pophelper: an R package and web app to analyse and visualize population structure. Molecular Ecology Resources 17:27–32

Frankham R (2009) Effective population size/adult population size ratios in wildlife: a review. Genetics Research 66:95−107

Frankham R, Bradshaw CJA, Brook BW (2014) Genetics in conservation management: Revised recommendations for the 50/500 rules, Red List criteria and population viability analyses. Biological Conservation 170:56−63

Funk WC, McKay JK, Hohenlohe, PA, Allendorf FW (2012) Harnessing genomics for delineating conservation units. Trends in Ecology and Evolution 27:489−496

Griffiths AM, Ellis JS, Clifton-Dey D, Machado-Schiaffino G, Bright D, Garcia-Vazquez E, Stevens JR (2011) Restoration versus recolonisation: the origin of Atlantic salmon (*Salmo salar* L.) currently in the River Thames. Biological Conservation 144:2733−2738

Gu Z (2014) Circlize implements and enhances circular visualization in R. Bioinformatics doi:10.1093/bioinformatics/btu393

Hazen L, Le Cornu E, Zerbe A, Martone R, Erickson AL, Crowder LB (2016) Translating sustainable seafood frameworks to assess the implementation of ecosystem-based fisheries management. Fisheries Research 182:149−157

Hemmer-Hansen J, Overgaard Therkildsen N, Pujolar JM (2014) Population genomics of marine fishes: next-generation prospects and challenges. Biological Bulletin 227:117−132

Heppell SA, Semmens BX, Pattengill-Semmens CV, Bush PG and others (2009) Tracking potential larval dispersal patterns from Nassau grouper aggregation sites: evidence for local retention and the 'importance of place'. Proceedings of the Gulf and Caribbean Fisheries Institute 61:325−327

Hess JE, Campbell NR, Close DA, Docker MF, Narum SR (2013) Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species. Molecular Ecology 22:2898–2916

Helyar SJ, Hemmer-Hansen J, Bekkevold D, Taylor MI, Ogden R, Limborg MT, Cariani A, Maes GE, Dioere E, Carvalho GR, Nielsen EE (2011) Application of

SNPs for population genetics of nonmodel organisms: new opportunities and challenges. Molecular Ecology Resources 11:123–136

Hohenlohe PA, Bassham S, Etter PD, Stiffler N, Johnson EA, Cresko WA (2010) Population genomics of parallel adaptation in threespine stickleback using sequenced RAD tags. PLoS Genetics 6:e1000862

Hughes TP, Bellwood DR, Folke CS, McCook LJ, Pandolfi JM (2007) No-take areas, herbivory and coral reef resilience. Tends in Ecology and Evolution 22:1−3

IUCN (2017) The IUCN red list of threatened species, Version 2017-1. http://www.iucnredlist.org/. Downloaded on 5 September 2017.

Jackson AM, Semmens BX, Sadovy de Mitcheson Y, Nemeth RS, Heppell SA, Bush PG, Aguilar-Perera A, Claydon JA, Calosso MC, Sealey KS, Scharer MT, Bernardi G (2014) Population structure and phylogeography in Nassau grouper (*Epinephelus striatus*), a mass-aggregating marine fish. PLoS One 9:e97508

Javid B, MacAry PA, Lehner PJ (2007) Structure and function: heat shock proteins and adaptive immunity. The Journal of Immunology 179:2035−2040

Jombart T (2008) adegenet: a R package for the multivariate analysis of genetic markers. Bioinformatics 24

Jombart T, Devillard S, Balloux F (2010) Discriminant analysis of principal components: a new method for the analysis of genetically structured populations. BMC Genetics 11:94, doi: 10.1186/1471-2156-11-94

Kadison E, Nemeth RS, Blondeau J, Smith T, Calnan J (2010) Nassau Grouper (*Epinephelus striatus*) in St. Thomas, US Virgin Islands, with evidence for a spawning aggregation site recovery. Proceedings of the Gulf and Caribbean Fisheries Institute 62:273−279

Kalinowski ST (2010) The computer program STRUCTURE does not reliably identify the main genetic clusters within species: simulations and implications for human population structure. Heredity 106:625–632

Kamvar ZN, Brooks JC, Grünwald NJ (2015) Novel R tools for analysis of genome-wide population genetic data with emphasis on clonality. Frontiers in Genetics 6:208

Kamvar ZN, Tabima JF, Grünwald NJ (2014) *Poppr*: an R package for genetic analysis of populations with clonal, partially clonal, and/or sexual reproduction. PeerJ 2:e281

Keenan K, McGinnity P, Cross TF, Crozier WW, Prodohl PA (2013) diveRsity: An R package for the estimation and exploration of population genetics parameters and their associated errors. Methods in Ecology and Evolution 4:782−788, doi:10.1111/2041-210X.12067

Kough AS, Paris CB (2015) The influence of spawning periodicity on population connectivity. Coral Reefs 34:753−757

Larson WA, Seeb LW, Everett MV, Waples RK, Templin WD, Seeb JE (2014) Genotyping by sequencing resolved shallow population structure to inform conservation of Chinook salmon (*Oncorhynchus tshawytscha*). Evolutionary Applications 7:355−369

Li Z, Srivastava P (2004) Heat-shock proteins. Current Protocols in Immunology 58, doi.org: 10.1002/0471142735.ima01ts58

Lipcius RN, Stockhausen WT, Eggleston DB (2013) Metapopulation dynamics and marine reserves: Caribbean spiny lobster in Exuma Sound, Bahamas. In (Chapter 7 The Bahamas: Tropical Island Nation) Marine Conservation (Eds. Ray, G.C. and J. McCormick-Ray). Blackwell Scientific, Oxford, UK

Liscjher HEL, Excoffier L (2012) PGDSpider: An automated data conversion tool for connecting population genetics and genomics programs. Bioinformatics 28:298−299

Lowe WH, Kovach RP, Allendorf FW (2017) Population genetics and demography unite ecology and evolution. Trends in Ecology and Evolution 32:141−152

Lorenzen K, Agnalt A-L, Blankenship HL, Hines AH, Leber KM, Loneragan NR, Taylor MD (2013) Evolving context and maturing science: aquaculture-based enhancement and restoration enter the marine fisheries management toolbox. Reviews in Fisheries Science 21:213−221

Manoukis NC (2007) Formatomatic: a program for converting diploid allelic data between common formats for population genetic analysis. Molecular Ecology Notes 7592−593

Mardis ER (2008) The impact of next-generation sequencing technology on genetics. Trends in Genetics 24:133−141

Martinsohn JT, Ogden R (2009) FishPopTrace—Developing SNP-based population genetic assignment methods to investigate illegal fishing. Forensic Science International: Genetics Supplement Series doi: /10.1016/j.fsigss.2009.08.108

McMahon BJ, Teeling EC, Höglund J (2014) How and why should we implement genomics into conservation? Evolutionary Applications 7:999−1007

Meirmans PG, Van Tienderen PH (2004) GENOTYPE and GENODIVE: two programs for the analysis of genetic diversity of asexual organisms. Molecular Ecology Notes 4:792−794

Miller AD, Van Rooyen A, Rašić G, Ierodiaconou DA, Gorfine HK, Day R, Wong C, Hoffmann AA, Weeks AR (2016) Contrasting patterns of population

connectivity between regions in a commercially important mollusc *Haliotis rubra*: integrating population genetics, genomics and marine LiDAR data. Molecular Ecology doi:10.1111/mec.13734

Molloy PP, Côté IM, Reynolds JD (2012) Why spawn in aggregations? In: Sadovy de Mitcheson Y, Colin PL (eds) Reef fish spawning aggregations: biology, research and management, Springer, New York, NY, p 57−84

Moore J-S, Harris LN, Le Luyer, J, Sutherland BJG, Rougemont Q, Tallman RF, Fisk AT, Bernatchez L (2017) Migration harshness drives habitat choice and local adaptation in anadromous Arctic char: evidence from integrating population genomics and acoustic telemetry. bioRxiv 10.1101/138545

Mumby PJ, Harborne AR (2010) Marine reserves enhance the recovery of corals on Caribbean reefs. PLoS ONE 5:e8657

Murchie KJ, Cooke SJ, Danylchuk AJ et al (2011) Thermal biology of bonefish (*Albula vulpes*) in Bahamian coastal waters and tidal creeks: an integrated laboratory and field study. Journal of Thermal Biology 36:38–48

Narum SR, Hess JE (2011) Comparison of F_{ST} outlier tests for SNP loci under selection. Molecular Ecology Resources 11:184–194

Nei M (1972) Genetic distance between populations. The American Naturalist. 106:283−292

Nomura T (2008) Estimation of effective number of breeders from molecular coancestry of single cohort sample. Evolutionary Applications 1:462−474

Ogden R, Gharbi K, Mugue N (2013) Sturgeon conservation genomics: SNP discovery and validation using RAD sequencing. Molecular Ecology 22:3112-3123

Olsen DA, LaPlace JA (1979) A study of a Virgin Islands grouper fishery based on a breeding aggregation. Proceedings of the Gulf and Caribbean Fisheries Institute 31:130−144

Overgaard Therkildsen N, Hemmer-Hansen J, Berg Hedeholm R, Wisz MS, Pampoulie C, Meldrup D, Bonanomi S, Retzel A, Malskær Olsen S, Eg Nielsen E (2013) Spatiotemporal SNP analysis reveals pronounced biocomplexity at the northern range margin of Atlantic cod *Gadus morhua*. Evolutionary Applications 6:690–705

Paris JR, Stevens, JR, Catchen JM (2017) Lost in parameter space: a road map for STACKS. Methods in Ecology and Evolution DOI: 10.1111/2041-210X.12775

Pauly D, Zeller D (2017) Comments on FAOs State of World Fisheries and Aquaculture (SOFIA 2016). Marine Policy 77:176−181

Perrier C, Guyomard R, Bagliniere J-L, Nikolic N, Evanno G (2013) Changes in the genetic structure of Atlantic salmon populations over four decades reveal substantial impacts of stocking and potential resiliency. Ecology and Evolution 3:2334−2349

Pittman SJ, Moaco ME, Friedlander AM, Legare B, Nemeth RS, Kendall MS, Poti M, Clark RD, Weding LM, Caldow C (2014) Fish with chips: tracking reef fish movements to evaluate size and connectivity of Caribbean marine protected areas. PLoS One 9:e96028

Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945−959

Pujolar JM, Jacobsen MW, Frydenberg J, Als TD, Larsen PF, Maes GE, Zane L, Jian JB, Cheng L, Hansen MM (2013) A resource of genome-wide single-nucleotide polymorphisms generated by RAD tag sequencing in the critically endangered European eel. Molecular Ecology Resources 13: 706–714

Rambaut A, Suchard M, Xie D, Drummond A (2014) Tracer v 1.6. Available at: http://beast.bio.ed.ac.uk/Tracer

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): Population genetics software for exact tests and ecumenicisim. Journal of Heredity 86:248−249

Reiss H, Hoarau G, Dickey-Collas M, Wolff WJ (2009) Genetic population structure of marine fish: mismatch between biological and fisheries management units. Fish and Fisheries 10:361−395

Rivera MAJ, Andrews KR, Kobayashi DR, Wren JLK, Kelley C, Roderick GK, Toonen RJ (2011) Genetic analyses and simulations of larval dispersal reveal distinct populations and directional connectivity across the range of the Hawaiian Grouper (*Epinephelus quernus*). Journal of Marine Biology 2011:1−11

Rousset F (2008) GENEPOP'007: a complete re-implementation of the GENEPOP software for Windows and Linux. Molecular Ecology Resources 8:103−106

Sadovy de Mitcheson Y, Colin PL (eds.) (2012) Reef Fish Spawning Aggregations: Biology, Research and Management. Vol. 35. Fish and Fisheries. Springer, New York

Sadovy de Mitcheson YS, Erisman B (2012) Fishery and biological implications of fishing spawning aggregations, and the social and economic importance of aggregating fishes. In: Sadovy de Mitcheson Y, Colin PL (eds) Reef fish spawning aggregations: biology, research and management. Springer, New York, NY, p 225−284

Sadovy de Mitcheson Y, Cornish A, Domeier M, Colin PL, Russell M, Lindeman KC (2008) A global baseline for spawning aggregations of reef fishes. Conservation Biology 22:1233−1244

Sadovy de Mitcheson Y, Craig MT, Bertoncini AA, Carpenter KE, Cheung WLL, Choat JH, Cornish AS, Fennessy ST, Ferreira BP, Heemstra PC, Liu M, Myers RF, Pollard DA, Rhodes KL, Rocha LA, Russell BC, Samoilys MA, Sanciangco J (2013) Fishing groupers towards extinction: a global assessment of threats and extinction risks in a billion dollar fishery. Fish and Fisheries 14:119−136

Seeb JE, Carvalho G, Hauser L, Naish K, Roberts S, Seeb LW (2011) Single-nucleotide polymorphism (SNP) discovery and applications of SNP genotyping in nonmodel organisms. Molecular Ecology Resources 11:1−8

Selkoe KA, D'Aloia CC, Crandall ED, Iacchei M, Liggins L, Puritz JB, von der Heyden S, Toonen RJ (2016) A decade of seascape genetics: contributions to basic and applied marine connectivity. Marine Ecology Progress Series 554:1−19

Semmens BX, Luke KE, Bush PG, Pattengill-Semmens C, Johnson B, McCoy C, Heppell S (2007) Investigating the reproductive migration and spatial ecology of Nassau grouper (*Epinephelus striatus*) on Little Cayman Island using acoustic tags — an overview. Proceedings of the Gulf and Caribbean Fisheries Institute 58: 199−206

Sherman KD, Dahlgren CP, Stevens JR, Tyler CR (2016) Integrating population biology into conservation management for endangered Nassau grouper *Epinephelus striatus*. Marine Ecology Progress Series 554:263−280

Sherman KD, King RA, Dahlgren CP, Simpson SD, Steven JR, Tyler CR (2017) Historic and contemporary anthropogenic activities influence genetic population dynamics of Nassau grouper (*Epinephelus striatus*) within The Bahamas. Frontiers in Marine Science 4:393. doi:10.3389/fmars.2017.00393

Sherman KD, Shultz AD, Dahlgren CP, Thomas C, Brooks E, Brooks A, Brumbaugh DR, Gittens L, Murchie K (In Press) Contemporary and emerging fisheries in The Bahamas –conservation and management challenges, achievements and future directions. Fisheries Management and Ecology doi: 10.1111/fme.12299

Shultz AD, Zuckerman ZC, Stewart HA, Suski CD (2014) Seasonal blood chemistry response of sub-tropical nearshore fishes to climate change. Conservation Physiology 2:1–12

Smith CL (1972) A spawning aggregation of Nassau grouper, *Epinephelus striatus* (Bloch). Transactions of the American Fisheries Society 101:257−261

Starr RM, Sala E, Ballesteros E, Zabala M (2007) Spatial dynamics of the Nassau grouper *Epinephelus striatus* in a Caribbean atoll. Marine Ecology Progress Series 343:239−249

Stoner A, Mueller K, Brown-Peterson N, Davis M, Booker CJ (2012) Maturation and age in queen conch (*Strombus gigas*): urgent need for changes in harvest criteria. Fisheries Research 131–133:76−84

Storey JD, Tibshirani R (2003) Statistical significance for genome-wide studies. Proceedings of the National Academy of Sciences USA 100:9440–9445

Stump K, Dahlgren CP, Sherman KD, Knapp CR (2017) Nassau grouper migrations during full moon suggest collapsed historic fish spawning aggregation and evidence of undocumented aggregation. Bulletin of Marine Science 93:375−389

Sunday JM, Calosi P, Dupont S, Munday PL, Stillman JH, Reusch TBH (2014) Evolution in an acidifying ocean. Trends in Ecology and Evolution 29:117−125

Tamura KG, Stecher G, Peterson D, Filipski A, Kumar S (2013) MEGA6: molecular evolutionary genetics analysis version 6.0. Molecular Biology and Evolution 30:2725−2729

Thurstan RH, McClenachan L, Crowder LB, Drew JA, Kittinger JN, Levin PS, Roberts CM, Pandolfi JM (2015) Filling historical data gaps to foster solutions in marine conservation. Ocean and Coastal Management 115:31−40

Vergara M, Basto MP, José Madeira M, Gómez-Moliner BJ, Santos-Reis M, Fernandes C, Ruiz-González A (2015) Inferring population genetic structure in widely and continuously distributed carnivores: the stone marten (*Martes foina*) as a case study. PLoS One, doi: /10.1371/journal.pone.0134257

Vidal O, García–Marín JL (2011) Ecological genetics of freshwater fish: a short review of the genotype–phenotype connection. Animal Biodiversity and Conservation 34.2: 309–317

Wang J, Xue D-X, Zhang B-D, Li, Y-L, Liu B-J (2016) Genome-wide SNP discovery, genotyping and their preliminary applications for population genetic inference in spotted sea bass (*Lateolabrax maculatus*). PLoS One 11: e0157809

Weir BS, Cockerham CC (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

Whaylen L, Bush PG, Johnson BC, Luke KE, McCoy C, Heppell S, Semmens B, Boardman M (2007) Aggregation dynamics and lessons learned from five years of monitoring at a Nassau grouper (*Epinephelus striatus*) spawning aggregation in Little Cayman, Cayman Islands, BWI. Proceedings of the Gulf and Caribbean Fisheries Institute 59:413−422

Whaylen L, Pattengill-Semmens CV, Semmens BX, Bush PG, Boardmand MR (2004) Observations of a Nassau grouper, *Epinephelus striatus*, spawning aggregation site in Little Cayman, Cayman Islands, including multispecies spawning information. Environmental Biology of Fishes 70:305−313

White JW (2015) Marine reserve design theory for species with ontogenetic migration. Biology Letters 11: 20140511, doi:10.1098/rsbl.2014.0511

Whiteley AR, Bhat A, Martins EP, Mayden RL, Arunachalam M, Uusi-HeikkilÄ S, Ahmed ATA, Shrestha J, Clark M, Stemple D, Bernatchez L (2011) Population genomics of wild and laboratory zebrafish (*Danio rerio*). Molecular Ecology 20:4259−4276

Wilson GA, Rannala B (2003) Bayesian inference of recent migration rates using multilocus genotypes. Genetics 163:1177−1191

Xu S, Song N, Zhao L, Cai S, Han Z, Gao T (2017) Genomic evidence for local adaptation in the ovoviviparous marine fish *Sebastiscus marmoratus* with a background of population homogeneity. Scientific Reports 7:1562, doi:10.1038/s41598-017-01742-z

Zhang B-D, Xue D-X, Wang J, Li Y-L, Liu B-J, Liu J-X (2016) Development and preliminary evaluation of a genomewide single nucleotide polymorphisms resource generated by RAD-seq for the small yellow croaker (*Larimichthys polyactis*). Molecular Ecology Resources 16: 755–768

Supplementary Materials

RAD-Seq analysis and *in situ* monitoring of Nassau grouper reveal fine-scale population structure and origins of aggregating fish

Krista D. Sherman, Josephine R. Paris, Karen A. Moore, R. Andrew King, Craig P. Dahlgren, Kristine Stump, Lindy C. Knowles, Jamie R. Stevens* and Charles R. Tyler*

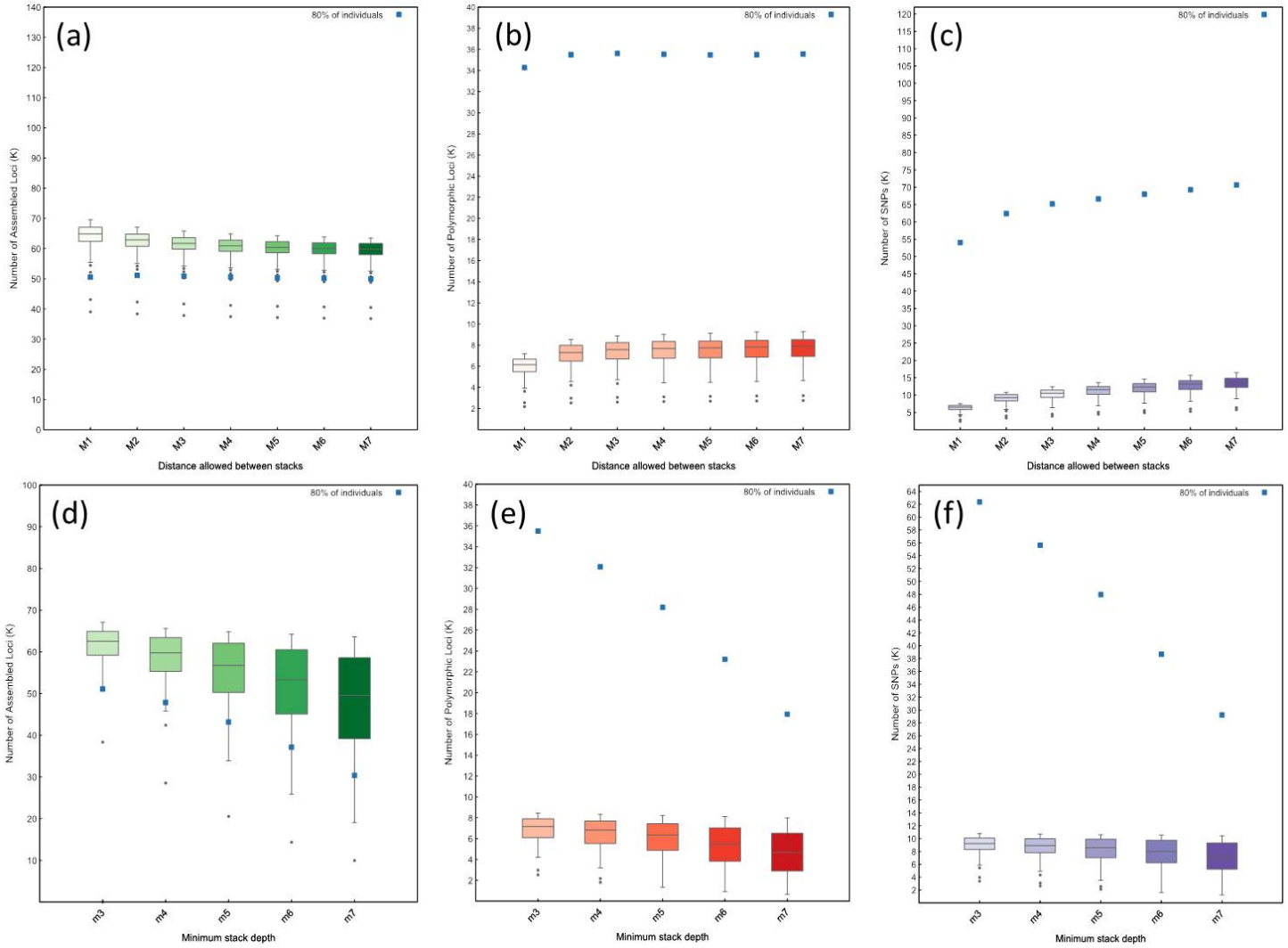
*Correspondence:
Dr. Jamie R. Stevens
J.R.Stevens@exeter.ac.uk

Professor Charles Tyler
C.R.Tyler@exeter.ac.uk

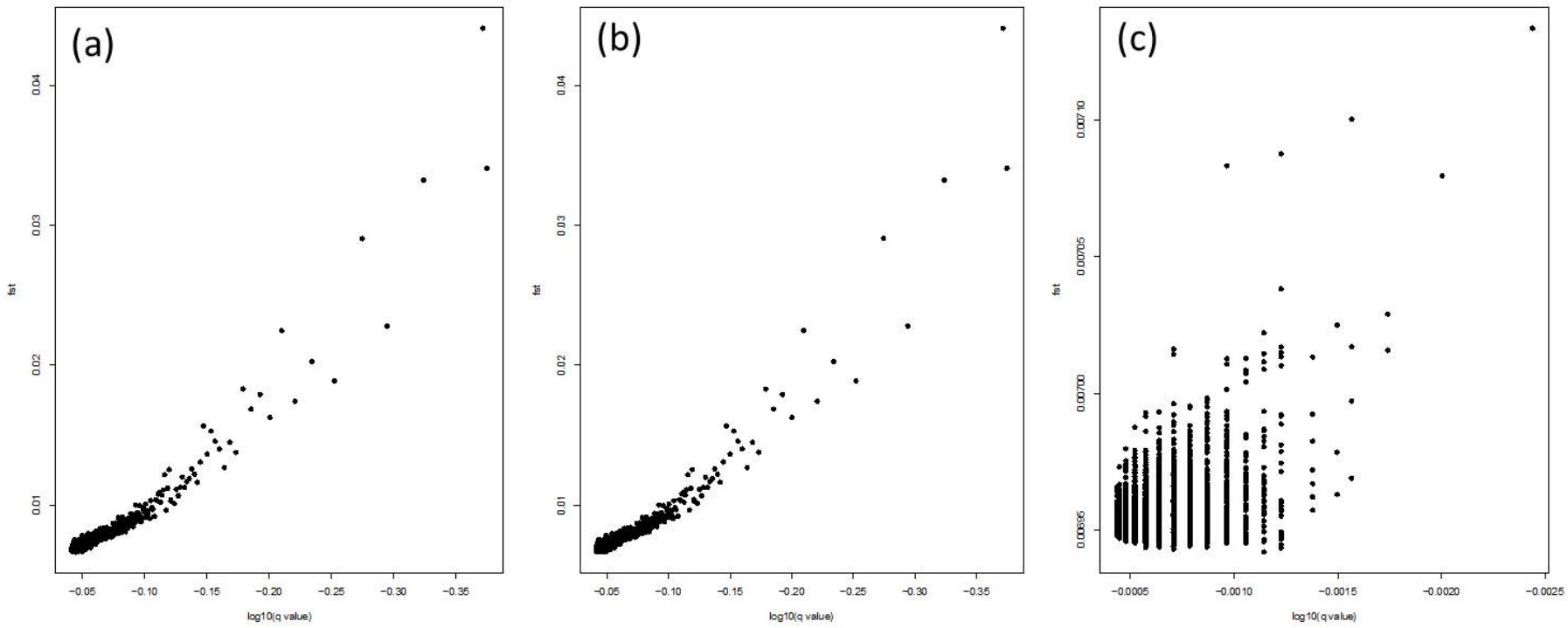
List of Figures

List of Tables

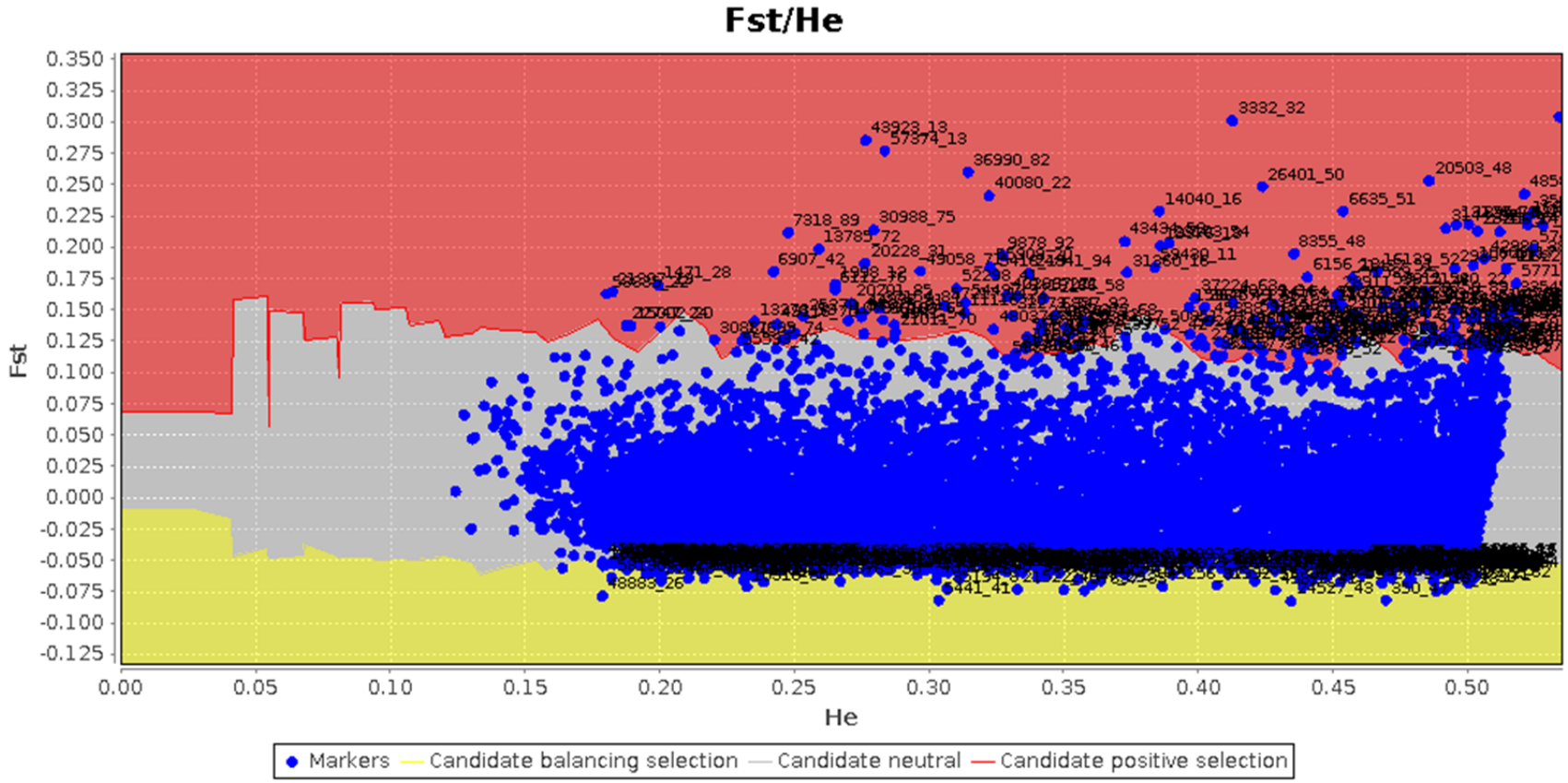
Supplementary Fig. 1



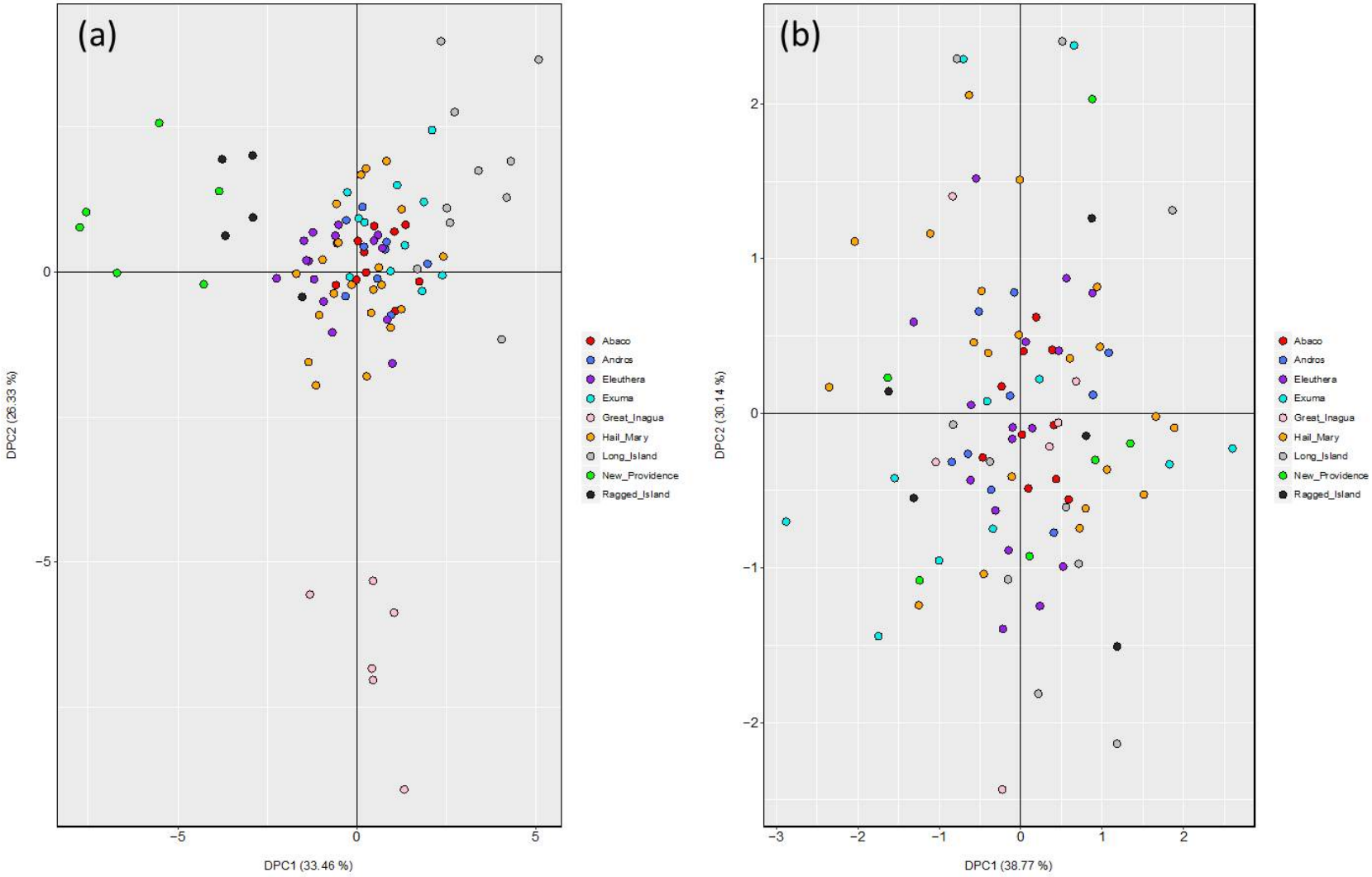
Supplementary Fig. 2.



Supplementary Fig. 3.



Supplementary Fig. 4.



Supplementary Table 1.

No. Samples	Sample ID	Total RAD tags	Ambiguous RAD tags	Retained Reads	% ambiguous	Low Quality	Ambiguous Barcodes
1	AB13	1314681	406892	907538	31	251	0
2	AB19	788355	232548	555665	29	142	0
3	AB28	1107748	327509	780019	30	220	0
4	AB34	1098254	309093	788923	28	238	0
5	AB38	1409097	402331	1006472	29	294	0
6	AB3	735484	200196	535126	27	162	0
7	AB42	1828205	532255	1295565	29	385	0
8	AB46	1471750	417650	1053837	28	263	0
9	AB5	862740	237837	624700	28	203	0
10	AB8	1109028	320901	787897	29	230	0
11	AN013	1275502	351111	924125	28	266	0
12	AN026	282444	80095	202292	28	57	0
13	AN075	1569115	462294	1106492	29	329	0
14	AN098	1524022	441820	1081888	29	314	0
15	AN151	1505077	422462	1082285	28	330	0
16	AN153	918726	272114	646440	30	172	0
17	AN161	1471706	405675	1065716	28	315	0
18	AN163	1196336	347039	849036	29	261	0
19	AN175	1856939	566345	1290208	30	386	0
20	AN192	913645	234487	678965	26	193	0
21	EL0931	1597171	415588	1181243	26	340	0
22	EL0976	1030338	301019	729099	29	220	0
23	EL0977	1931350	527575	1403347	27	428	0
24	EL0982	2375488	761712	1613331	32	445	0
25	EL0984	1004119	288465	715444	29	210	0
26	EL0989	2062402	567957	1494005	28	440	0
27	EL0990	1971954	535226	1436306	27	422	0
28	EL0991	2043706	542723	1500575	27	408	0
29	EL0993	1416980	388901	1027775	27	304	0
30	EL0997	2177744	613678	1563601	28	465	0

31	EL0999	3158765	880392	2277758	28	615	0
32	EL1000	527484	147157	380234	28	93	0
33	EL11	1246371	367749	878342	30	280	0
34	EL3	969452	263685	705570	27	197	0
35	EL4	1715290	470149	1244795	27	346	0
36	ELJV1	1356429	382821	973332	28	276	0
37	EX0501	2395979	705547	1689959	29	473	0
38	EX0513	1921761	548650	1372701	29	410	0
39	EX0518	1927808	527083	1400295	27	430	0
40	EX0525	2221825	647913	1573443	29	469	0
41	EX0526	1539831	433113	1106381	28	337	0
42	EX0547	2514922	765439	1748895	30	588	0
43	EX0548	2032772	567032	1465311	28	429	0
44	EX112	1736712	526582	1209796	30	334	0
45	EX116	1908356	530622	1377359	28	375	0
46	EX187	1578324	454350	1123634	29	340	0
47	EXJV1	3219147	906663	2311788	28	696	0
48	GI12	2230560	641786	1588296	29	478	0
49	GI16	1319065	360824	957961	27	280	0
50	GI19	992334	268015	724121	27	198	0
51	GI24	836986	225519	611304	27	163	0
52	GI33	1231369	345803	885307	28	259	0
53	GI8	1323234	378926	944039	29	269	0
54	LI102	1273966	345853	927825	27	288	0
55	LI118	1319768	364464	955050	28	254	0
56	LI201	1196682	336692	859724	28	266	0
57	LI203	1253014	349854	902902	28	258	0
58	LI205	1419372	404111	1014974	28	287	0
59	LI212	1665769	451398	1214013	27	358	0
60	LI213	2734799	775289	1958951	28	559	0
61	LI215	1373743	372032	1001422	27	289	0
62	LI217	1666033	462420	1203278	28	335	0
63	LI222	1743350	479401	1263571	27	378	0
64	LI223	2724067	750694	1972795	28	578	0
65	LI226	2151708	663889	1487408	31	411	0

66	LI227	3653394	1060001	2592645	29	748	0
67	LI228	1775597	492477	1282769	28	351	0
68	LI231	2808905	844351	1963960	30	594	0
69	LI234	2631815	768935	1862355	29	525	0
70	LI237	1203786	335078	868477	28	231	0
71	LI239	272483	75186	197243	28	54	0
72	LI242	1094286	297505	796571	27	210	0
73	LI244	1723430	477970	1245110	28	350	0
74	LI253	2812783	773117	2039046	27	620	0
75	LI254	1053167	286632	766292	27	243	0
76	LI255	1449603	406121	1043182	28	300	0
77	LI257	2300855	660646	1639726	29	483	0
78	LI259	2612357	711045	1900772	27	540	0
79	LI265	1912304	540450	1371477	28	377	0
80	LI269	2186065	625163	1560472	29	430	0
81	LI271	2247749	610331	1636938	27	480	0
82	LI272	2893701	816431	2076651	28	619	0
83	LIJVCB1	2578809	699526	1878707	27	576	0
84	LIJVCB2	1371154	359620	1011216	26	318	0
85	LIJVCB4	1196735	322706	873793	27	236	0
86	NP11	1729291	502841	1226078	29	372	0
87	NP12	1898172	531159	1366618	28	395	0
88	NP13	1030403	287762	742424	28	217	0
89	NP36	2712408	742166	1969680	27	562	0
90	NP37	1230906	332175	898467	27	264	0
91	NPRI7	897314	241586	655519	27	209	0
92	RI1	2456379	715934	1739954	29	491	0
93	RI2	473878	134010	339761	28	107	0
94	RI3	1475903	410094	1065522	28	287	0
95	RI4	2068607	593490	1474698	29	419	0
96	RI7	1166568	318588	847737	27	243	0
Total		**159,195,960**	**44,988,511**	**114,174,309**	—	**33,140**	—
Min		272,483	75,186	197,243	—	54	—
Max		3,653,394	1,060,001	259,2645	—	748	—
Mean		**1658291.25**	**468,630.32**	**1,189,315.72**	—	**345.21**	—

STDEV	671,515.34	194,452.85	478,376.18	—	141.30	—

Supplementary Table 2.

No. of Samples	Transmitter No.	Sample ID	Location	No. assembled loci	No. Polymorphic loci	No. SNPs	Depth of Coverage	No. of heterozygous SNPs
1	44313	AB13	Abaco	54740	6144	8902	13	28
2	44318	AB19	Abaco	36467	2695	4371	9	26
3	44320	AB28	Abaco	47999	4640	7022	11	285
4	None	AB34	Abaco	50489	4929	7361	12	39
5	None	AB38	Abaco	54905	6452	9262	15	1442
6	44307	AB3	Abaco	33407	2480	4115	10	65
7	None	AB42	Abaco	59612	7697	10710	18	1507
8	None	AB46	Abaco	55764	6488	9465	14	62
9	44303	AB5	Abaco	39530	3229	5144	10	51
10	44306	AB8	Abaco	51167	5176	7690	12	148
11	57259	AN013	Andros	53315	5942	8491	14	213
12	57257	AN075	Andros	55268	6503	9328	15	546
13	57261	AN098	Andros	57870	7005	9923	15	1131
14	13664	AN151	Andros	55737	6518	9283	16	1532
15	13666	AN153	Andros	40272	3501	5437	11	51
16	13672	AN161	Andros	55549	6563	9378	16	752
17	13663	AN163	Andros	48630	5089	7376	13	201
18	13670	AN175	Andros	60926	7964	11056	19	505
19	13676	AN192	Andros	42768	3964	5945	12	259
20	None	EL0931	Eleuthera	55912	6670	9544	17	488
21	None	EL0976	Eleuthera	44084	4160	6299	12	1820
22	None	EL0977	Eleuthera	59848	7606	10645	20	933
23	None	EL0982	Eleuthera	61369	8094	11304	22	592
24	None	EL0984	Eleuthera	45874	4437	6665	12	43
25	None	EL0989	Eleuthera	59849	7594	10754	20	511
26	None	EL0990	Eleuthera	58906	7363	10380	20	399
27	None	EL0991	Eleuthera	60444	7756	10812	21	483
28	None	EL0993	Eleuthera	53267	6069	8627	16	333
29	None	EL0997	Eleuthera	61930	8294	11502	22	757
30	None	EL0999	Eleuthera	63141	8504	11986	30	803

31	None	EL1000	Eleuthera	24434	1713	3022	9	48
32	None	EL11	Eleuthera	51294	5555	7971	13	95
33	None	EL3	Eleuthera	47885	4765	6984	12	53
34	None	EL4	Eleuthera	57989	7162	10089	18	454
35	None	ELJV1	Eleuthera	54600	6215	8843	14	76
36	None	EX0501	Exuma	61865	8228	11430	24	442
37	None	EX0513	Exuma	60143	7802	10872	19	1093
38	None	EX0518	Exuma	58711	7410	10383	20	542
39	None	EX0525	Exuma	61405	8070	11217	22	3700
40	None	EX0526	Exuma	57512	7015	9881	16	1033
41	None	EX0547	Exuma	62164	8397	11652	24	466
42	None	EX0548	Exuma	60534	7764	10945	20	437
43	None	EX112	Exuma	60227	7755	10809	17	1299
44	None	EX116	Exuma	60239	7771	10850	19	7178
45	None	EX187	Exuma	58986	7287	10295	16	472
46	None	EXJV1	Exuma	62897	8496	11801	32	653
47	None	GI12	Great Inagua	61677	8204	11370	22	1452
48	None	GI16	Great Inagua	55574	6437	9092	14	135
49	None	GI19	Great Inagua	46425	4505	6703	12	19
50	None	GI24	Great Inagua	42032	3727	5776	11	128
51	None	GI33	Great Inagua	54817	6160	8781	13	413
52	None	GI8	Great Inagua	55483	6310	8892	14	133
53	57268	LI102	Hail Mary	48785	5195	7640	14	3056
54	57269	LI118	Hail Mary	51616	5787	8396	15	613
55	57265	LI201	Hail Mary	52754	5606	8248	13	116
56	None	LI203	Long Island	53787	5941	8531	14	60
57	None	LI205	Long Island	55491	6397	9086	18	364
58	13675	LI212	Hail Mary	57270	6862	9692	27	418
59	None	LI213	Hail Mary	61257	8238	11483	15	956
60	57266	LI215	Hail Mary	54457	6204	8898	18	8216
61	None	LI217	Long Island	58536	7234	10109	20	504
62	57264	LI222	Hail Mary	55833	6773	9787	34	928
63	None	LI223	Long Island	62359	8337	11581	18	2924
64	15791	LI226	Hail Mary	61905	8243	11443	26	463
65	15790	LI227	Hail Mary	63594	8687	12139	13	854

66	57284	LI228	Hail Mary	58808	7345	10374	17	1340
67	None	LI231	Long Island	62966	8394	11608	28	586
68	57278	LI234	Hail Mary	62441	8291	11493	12	447
69	None	LI237	Long Island	51469	5451	7717	15	141
70	57282	LI242	Hail Mary	45954	4563	6722	22	133
71	None	LI244	Hail Mary	58028	7122	9959	20	3722
72	10264	LI253	Hail Mary	62158	8333	11726	22	621
73	57277	LI254	Hail Mary	48329	4906	7284	29	25
74	10274	LI255	Hail Mary	55707	6524	9220	15	763
75	10272	LI257	Hail Mary	61682	8250	11468	18	494
76	15788	LI259	Hail Mary	61787	8044	11258	27	1884
77	10273	LI265	Hail Mary	59912	7742	10733	27	699
78	57263	LI269	Long Island	61062	8020	11248	13	687
79	10266	LI271	Hail Mary	60875	8171	11410	26	658
80	15789	LI272	Hail Mary	61914	8117	11410	21	417
81	None	LIJVCB1	Long Island	61637	8007	11168	26	917
82	None	LIJVCB2	Long Island	54387	6151	8791	15	708
83	None	LIJVCB4	Long Island	52014	5525	7948	13	1097
84	None	NP11	New Providence	58411	7018	9924	17	697
85	None	NP12	New Providence	60229	7868	10976	20	424
86	None	NP13	New Providence	49776	4969	7267	12	1355
87	None	NP36	New Providence	62950	8418	11779	26	1167
88	None	NP37	New Providence	49288	5293	7626	14	127
89	None	NPRI7	New Providence	39273	3569	5504	11	91
90	None	RI1	Ragged Island	62301	8231	11374	24	462
91	None	RI2	Ragged Island	20298	1382	2489	9	600
92	None	RI3	Ragged Island	56636	6851	9612	16	1840
93	None	RI4	Ragged Island	61244	8057	11193	21	628
94	None	RI7	Ragged Island	44185	4561	6926	13	87
Total				**5,139,297**	**610,951**	**869,675**		**78,615**

Min	20,298	1,382	2489	9	19
Max	63,594	8687	12139	34	8216
Mean	**54,673.37**	**6,499.47**	**9,251.86**	**17**	**836.33**
STDEV	8,316.81	1,695.22	2,172.84	6	1,253.21

Supplementary Table 3.

Locus ID	Coding region/distance to nearest coding region and genes	Function	Nucleotide Diversity									
			AB	AN	EL	EX	GI	HM	LI	NP	RI	RI
1618	Damage specific DNA binding protein (ddb1)	DNA damage repair	0.305	0.305	0.492	0.298	0.278	0.482	0.420	0.486	0.375	0.375
1735	Calcium voltage-gated channel (cacna1s)	Calcium transport	0.117	0.475	0.459	0.484	—	0.488	0.420	0.444	—	—
1998	retrotransposon:BEL32-LTR DR, retrotransposon:BEL32-I DR, transposon:piggyBac-N3 DR, LINE:Expander, LINE:Bridge2(Xena), LINE:Rex1 FurC, clone: 180O20	Transposition, Ferric uptake	—	—	0	0.095	0.500	0.198	0.198	—	0.219	0.219
	retransposable elements	Gene regulation	—	0.346	0.404	0.434	0.444	0.469	0.500	0.000	0.320	—
	uncharacterized LOC107835383 (LOC107835383), ncRNA	Gene expression regulation	0.305	0.500	0.430	0.434	0.375	0.420	0.495	0.444	0.320	—
3144	zinc binding - zinc finger homeobox protein 3-like, transcript variant mRNA (LOC104918143)	Regulates myogenic and neuronal differentiation	—	0.494	0.408	0.397	0.375	0.495	0.500	0.444	0.219	0.320
4343	neuropilin-1a-like transcript variant mRNA (LOC109967076)	Cell migration	—	0.401	0.480	0.463	—	—	0.320	0.375	0.219	0.320

	neuropilin-1a-like transcript variant mRNA (LOC109989895)	Cell migration	0.180	0.105	0.219	0.087	0.153	0.172	0.180	0.278	0.480	—
	neuropilin-1a-like transcript variant mRNA (LOC104934053)	Cell migration	0.375	0.346	0.477	0.236	0.375	0.388	0.375	0.278	0.219	—
4748	protein FAM117A-like mRNA (LOC104946771)	Protein coding gene	0	0.444	0.219	0.000	0.153	0.091	0.255	0.278	0.500	0.219
5595	collagen alpha-1(XXVIII) chain (LOC104939025), mRNA (LOC104939025, LOC109998694, LOC104951329)	Cell adhesion	—	—	—	0.495	0	0	0.492	0.000	—	0.219
6112	protein-methionine sulfoxide oxidase mical3a-like, transcript variant mRNA (LOC109961790, LOC108875448, LOC105018133)	Metal ion binding, Exocytosis	—	0.500	0.191	0.434	—	0.346	0.278	—	0.000	0.480
6156	jumonji domain containing 1C (jmjd1c), mRNA	histone demethylation, regulation of transcription	0.000	0.105	0.320	0.420	0.375	0.210	0.180	0.500	0.000	0.219
	probable JmjC domain-containing histone demethylation protein 2C (LOC104955568), partial mRNA	histone demethylation, metal binding	0.305	0.492	0.464	0.434	0.444	0.495	0.255	0.000	0.375	—
10799	chromosome sequence corresponding to linkage group 18, complete sequence	~	0	—	0.337	0	0.486	0.245	0.500	0.153	0.320	0.500
	clusterin like 1 (clul1), transcript variant mRNA	Extracellecular protein - involved in neurodegeneration	0.000	0.198	0.064	0.255	0.500	0.289	0.375	0.153	0.000	—

	clusterin-like protein 1, mRNA (LOC110946200)	Extracellecular protein - involved in neurodegeneration	—	—	—	0.401	—	0.493	0.219	0.180	0.469	—
10983	*Epinephelus coioides* x *Epinephelus lanceolatus* voucher ECEL001 microsatellite ECELEB009 sequence	~	—	—	0.497	0.500	—	0.498	0.401	—	0.000	—
13453	Interferon alpha 1-like gene, complete sequence; growth hormone 1 gene, complete cds; and skeletal muscle sodium channel alpha subunit-like, myosin alkali light chain-like, and microtubule-associated protein Tau-like genes	Immune response, growth, muscle function,	0.000	0.117	0.000	0.298	—	0.198	0.480	0.180	—	0.000
	transposase (pG-ON6-9 transposon Tc1-like)	~	—	0.492	0.469	0.434	0.444	0.499	0.455	0.278	0.219	—
	SSTN12 tn gene for putative transposase	~	—	0.401	—	0.105	—	0.139	—	0.480	0.000	—
	heat shock protein gene (HSP70)	Heat stress/immune response	—	0.494	—	0.444	0.153	0.450	0.305	0.153	0.000	—
21011	semaphorin-3D-like (LOC102311894), transcript variant mRNA	Regulation of developmental processes	—	—	—	0.087	0.500	0.057	0.455	0.000	0	0.000
21034	integrin alpha-M-like, mRNA (LOC108237264)	Cell adhesion	0.375	—	0.453	0.484	0.486	0.399	0.401	1	0.219	0.375
22628	leucine rich repeat containing G protein-coupled receptor 5 (lgr5), mRNA	Protein hormone receptor	0.095	0.198	0.180	0.165	0.500	0.133	0.255	0.000	0.500	0.320

25270	strain HNI chromosome 15, Hd-rR chromosome 15 sequence and complete genome assembly, strain HSOK chromosome 15	Cell differentiation, sexual differentiation, transcription regulation, metal-binding, DNA binding	0	0.198	0.320	0.351	0.278	0.375	0.375	0.278	0	0.000
28915	nuclear factor 1 X-type-like, partial mRNA (LOC109644827)	DNA replication, transcription regulation	—	—	0.436	0.298	0.480	0.387	0.255	0.320	0.375	0.469
	nuclear factor I X (nfix), transcript variant X10, mRNA	DNA replication, transcription regulation	—	—	—	0.401	0.000	0.320	—	0.000	0.000	—
	nuclear factor 1 X-type-like, transcript variant X10, mRNA (LOC110955692)	DNA replication, transcription regulation	—	0.375	—	0.484	—	0.450	0.219	0.180	—	—
29678	*Epinephelus fuscoguttatus* microsatellite EFJ007 sequence	~	—	0.305	—	0.165	0.486	0.349	0.320	—	—	0.000
	clone 124-1 epinecidin 1 gene, promoter region	Immune response										
30881	actin related protein 2 homolog mRNA (actr2)	Complex-mediated actin nucleation, meiotic cytokinesis	0	0.117	0	0.298	—	0.198	0.480	0.180	—	—
	actin related protein 2-A (LOC104930021)	Complex-mediated actin nucleation, meiotic cytokinesis										
31184	solute carrier family 13 (sodium-dependent citrate transporter), member 5, mRNA (slc13a5)	citrate transport, tricarboxylic acid transmembrane transport	—	0.492	0.469	0.434	0.444	0.499	0.455	0.278	0.219	0.219
36189	collagen alpha-1(XI) chain-like, transcript variant, mRNA (LOC109629802)	Extracellular matrix binding, metal ion binding, protein binding	—	0.401	—	0.105	—	0.139	—	0.480	0	0.000

40496	ST3 beta-galactoside alpha-2,3-sialyltransferase 4 (st3gal4), transcript variant, mRNA	Oligosaccharide metabolic processes	—	0.494	—	0.444	0.153	0.450	0.305	0.153	0	0.000
43923	chromosome sequence corresponding to linkage group 18, complete sequence	~	—	—	—	0.087	0.500	0.057	0.455	0	0	0.000
	strain HSOK chromosome 20	~										
	strain HNI chromosome 20	Metal ion binding, transcription factor activity, sequence-specific DNA binding										
45856	protein piccolo-like, transcript variant, mRNA (LOC108875125)	Presynaptic cytoskeletal matrix: involved with synaptic zones	0.375	—	0.453	0.484	0.486	0.399	0.401	0.500	0.219	0.219
49058	clone Pf_1879 ribosome-binding protein 1 (Rrbp1) mRNA, partial cds	Protein transport, translation	0.095	0.198	0.180	0.165	0.500	0.133	0.255	0	0.500	0.500
50851	kin of IRRE like 2, transcript variant, mRNA (kirrel2)	Brain development, glomerular development, myoblast fusion, pronephric duct and glomerulus development	0.278	0.198	0.320	0.351	0.278	0.375	0.375	0.278	0.375	0.375
58317	chromosome sequence corresponding to linkage group 1, top part, complete sequence	~	—	—	0.436	0.298	0.480	0.387	0.255	0.320	0.375	0.375
	microsatellite Luer6 sequence	~										

58835	succinate dehydrogenase complex assembly factor 1 (sdhaf1), mRNA	mitochondrial respiratory chain complex II assembly, musculoskeletal movement, regulation of gluceneogensis, response to hyperoxia & oxidative stress, succinate metabolic processes	—	—	—	0.401	0	0.320	—	0	0	0.000
59159	common carp genome, scaffold: LG28, chromosome: 28	~	—	0.375	—	0.484	—	0.450	0.219	0.180	—	—
	clone CH211-129M12 in linkage group 13, complete sequence	~										
	clone DKEYP-2B10 in linkage group 7, complete sequence	~										
59242	THUMP domain containing 2, transcript variant, mRNA (thumpd2)	RNA binding	—	0.305	—	0.165	0.486	0.349	0.320	—	—	—

Supplementary Table 4.

Locus ID	Coding region/distance to nearest coding region and genes	Top Species Hits	Top Species Common Names	No. SNPs	H_E	H_O	π	F_{IS}	Query Cover (%)	Identity Score (%)
350	solute carrier family 35 member B4 (slc35b4), mRNA	*Lates calcarifer, Larimichthys crocea, Notothenia coriiceps*	barramundi, large yellow croaker, black rockcod	1	0.283	0.464	0.508	0.441	87	94-95%
	HSOK chromosome 6	*Oryzias latipes*	Japanese rice fish						93	90
	UDP-xylose and UDP-N-acetylglucosamine transporter								87	90
458	exostosin-like glycosyltransferase 3 (extl3), mRNA	*Notothenia coriiceps, Monopterus albus, Larimichthys crocea*	black rockcod, Asian swamp eel, large yellow croaker	1	0.220	0.194	0.207	-0.056	90-95	91-95
4033	coiled-coil domain-containing protein 63-like mRNA	*Paralichthys olivaceus*	olive flounder	1	0.265	0.236	0.254	-0.030	76	88
	Hd-rR chromosome 14 sequence	*Oryzias latipes*	Japanese rice fish						47	89

	strain Hd-rR, complete genome assembly, chromosome 14	*Oryzias latipes*	Japanese rice fish						47	89
5902	RNA-binding protein MEX3B (LOC104929649), mRNA	*Larimichthys crocea*	large yellow croaker	4	0.746	0.459	0.496	-0.494	100	90
13729	RNA guanylyltransferase and 5'-phosphatase (rngtt), transcript variant X2, mRNA	*Lates calcarifer, Fundulus heteroclitus, Labrus bergylta*	barramundi, mummichog, ballan wrasse	1	0.269	0.237	0.254	-0.039	95	90-92
	mRNA-capping enzyme-like (LOC102797251), mRNA	*Neolamprologus brichardi*	African cichlid						95	90
14566	family with sequence similarity 129 member A (fam129a), mRNA	*Lates calcarifer*	barramundi	3	0.293	0.436	0.471	0.374	96	89
	protein Niban-like (LOC109999805), mRNA	*Labrus bergylta*	ballan wrasse						87	90
	strain HSOK chromosome 4	*Oryzias latipes*	Japanese rice fish						58	96

14987	large (Drosophila) homolog-associated protein 5 (dlgap5), transcript variant X2, mRNA	*Haplochromis burtoni, Pundamilia nyererei, Maylandia zebra*	African cichlid, Victorian cichlid, Lake Malawi cichlid	2	0.521	0.487	0.522	0.003	100	94
15688	copine-8 (cpne8) transcript variant, mRNA	*Lates calcarifer, Larimichthys crocea, Nothobranchius furzeri*	barramundi, large yellow croaker, turquoise killifish	4	0.240	0.243	0.259	0.091	100	96-97
16572	cadherin-related family (cdhr1) member 1-like transcript variant, mRNA	*Paralichthys olivaceus, Lates calcarifer, Kryptolebias marmoratus*	olive flounder, barramundi, mangrove rivulus	1	0.384	0.365	0.391	0.006	100	93-94
17242	nucleoporin 98 (nup98) transcript variant, mRNA	*Acanthochromis polyacanthus, Esox Lucius*	spiny chromis damselfish, northern pike	1	0.211	0.194	0.209	0.026	29-65	90-100
18034	nesprin-2-like (LOC109988425), mRNA	*Labrus bergylta*	ballan wrasse	3	0.351	0.408	0.434	0.185	100	93
19666	neutral ceramidase-like (LOC108892356), mRNA	*Lates calcarifer*	barramundi	2	0.154	0.233	0.248	0.288	70	90

	N-acylsphingosine amidohydrolase 2 (asah2), transcript variant X3, mRNA	*Lates calcarifer*	barramundi						70	90
19897	contig00136 genomic sequence	*Sparus aurata*	gilt-head bream	5	0.288	0.384	0.410	0.304	59	89
	contig00151 genomic sequence	*Sparus aurata*	gilt-head bream						55	91
20385	major facilitator superfamily (mfsd8) domain-containing protein 8-like (LOC109636640), mRNA	*Paralichthys olivaceus, Notothenia coriiceps*	olive flounder, black rockcod	2	0.210	0.186	0.198	-0.051	83	96
	uncharacterized LOC104927507 (LOC104927507), mRNA	*Larimichthys crocea*	large yellow croaker						83	95
20427	strain Hd-rR chromosome 22 sequence	*Oryzias latipes*	Japanese rice fish	1	0.294	0.274	0.294	0.002	45	98
21724	prostaglandin E synthase 2 (ptges2), mRNA	*Lates calcarifer, Paralichthys olivaceus, Kryptolebias marmoratus*	barramundi, olive flounder, mangrove rivulus	3	0.251	0.359	0.387	0.315	100	95-98
	clone CFW204-C11 mRNA sequence	*Gasterosteus aculeatus*	three-spined stickleback						100	96

22878	chromosome sequence corresponding to linkage group 1, top part, complete sequence	*Dicentrarchus labrax*	European bass	1	0.286	0.463	0.497	0.430	48	96
24552	pleckstrin homology and RhoGEF domain containing G4B (plekhg4b), transcript variant X3, mRNA	*Labrus bergylta, Takifugu rubripes*	ballan wrasse, Japanese puffer	1	0.366	0.454	0.482	0.254	53-98	84-92
24909	strain HSOK chromosome 14	*Oryzias latipes*	Japanese rice fish	1	0.428	0.465	0.495	0.135	58	96
	MAP/microtubule affinity-regulating kinase 4-like (LOC110958165), transcript variant X2, mRNA	*Acanthochromis polyacanthus, Fundulus heteroclitus*	spiny chromis damselfish, mummichog						51	100
30972	nibrin (nbn), transcript variant X2, mRNA	*Lates calcarifer*	barramundi	1	0.583	0.498	0.534	-0.096	96	87

32035	hippocampus abundant transcript 1 protein-like (LOC108897265), transcript variant X2, mRNA	*Lates calcarifer*	barramundi	1	0.455	0.494	0.528	0.134	41	98
34598	histone-lysine N-methyltransferase SETD1B-A-like (LOC104931165), transcript variant X5, mRNA	*Larimichthys crocea, Paralichthys olivaceus, Lates calcarifer*	large yellow croaker, olive flounder, barramundi	3	0.251	0.233	0.247	-0.003	95-97	93-95
	SET domain containing 1B (Setd1b), mRNA	*Cavia porcellus*	guinea pig						93	86
34773	GTPase HRas-like (LOC109998395), mRNA	*Labrus bergylta*	ballan wrasse	2	0.391	0.329	0.352	-0.096	82	86
	genome assembly common carp genome, scaffold: LG38, chromosome: 38	*Cyprinus carpio*	common carp						71	87
36230	DnaJ heat shock protein family (Hsp40) member B5 (dnajb5), mRNA	*Astyanax mexicanus*	Mexican tetra	3	0.676	0.450	0.482	-0.397	37	97

44669	transmembrane protein 236-like (LOC108883272), mRNA	*Lates calcarifer*	barramundi	1	0.205	0.222	0.240	0.111	59	89
44727	signal induced proliferation associated 1 like 3 (sipa1l3), mRNA	*Paralichthys olivaceus, Larimichthys crocea, Acanthochromis polyacanthus*	olive flounder, large yellow croaker, spiny chromis damselfish	1	0.298	0.274	0.293	-0.001	96-100	91-94
46884	chromosome sequence corresponding to linkage group 1, top part, complete sequence	*Dicentrarchus labrax*	European bass	2	0.221	0.204	0.217	-0.027	96	87
47816	solute carrier family 12 member 5 (slc12a5), transcript variant X2, mRNA	*Larimichthys crocea, Monopterus albus, Lates calcarifer*	large yellow croaker, Asian swamp eel, barramundi	1	0.524	0.495	0.528	0.007	97-100	93-96
	strain Hd-rR chromosome 5 sequence	*Oryzias latipes*	japanese rice fish						100	94
50252	CUB and zona pellucida-like domain-containing protein 1 (LOC104939594), mRNA	*Larimichthys crocea*	large yellow croaker	2	0.282	0.241	0.255	-0.097	50	96

50345	chromosome sequence corresponding to linkage group 18, complete sequence	*Dicentrarchus labrax*	European bass	4	0.253	0.229	0.246	-0.020	92	93
51124	DnaJ heat shock protein family (Hsp40) member B5 (dnajb5), mRNA	*Astyanax mexicanus*	Mexican tetra	3	0.218	0.224	0.241	0.049	43	95
51751	growth/differentiation factor 11-like (LOC109141439), partial mRNA	*Larimichthys crocea, Lates calcarifer*	large yellow croaker, barramundi	1	0.189	0.193	0.206	0.073	75	89-96
52092	neurogenic locus notch homolog protein 1-like, mRNA (LOC108875238,LOC106528756)	*Lates calcarifer, Austrofundulus limnaeus*	barramundi, cypronid fish	2					93-100	90-92
	notch 1 (notch1), mRNA	*Labrus bergylta*	ballan wrasse						97	90
54277	tripartite motif-containing protein 16-like, mRNA (LOC106676446, LOC110001700, LOC109203666)	*Maylandia zebra, Labrus bergylta, Oreochromis niloticus*	Lake Malawi cichlid, ballan wrasse, Nile tilapia	7	0.675	0.441	0.475	-0.413	81-100	88-92

55709	solute carrier family 43 member 3 (slc43a3), mRNA (LOC105932669)	*Lates calcarifer, Fundulus heteroclitus, Monopterus albus*	barramundi, mummichog, Asian swamp eel	3	0.338	0.455	0.488	0.309	100	94-96
58982	tetratricopeptide repeat protein 7A-like, partial mRNA (LOC104943395, LOC108895692)	*Notothenia coriiceps, Lates calcarifer*	black rockcod, barramundi	2	0.206	0.183	0.195	-0.047	98	91-91
	uncharacterized LOC103386891 (LOC103386891), transcript variant X2, ncRNA	*Monopterus albus*	Asian swam eel						98	89
	tetratricopeptide repeat domain 7A (ttc7a), mRNA	*Labrus bergylta*	ballan wrasse						98	88
59223	ankyrin-1-like, transcript variant mRNA (LOC110963983, LOC104941581)	*Acanthochromis polyacanthus, Notothenia coriiceps*	spiny chromis damselfish, black rockcod	2					59	95
	ankyrin 1 (ank1), transcript variant X3, mRNA	*Paralichthys olivaceus*	olive flounder						59	95

Supplementary Table 5.

Locus ID	Coding region/distance to nearest coding region and genes	Function	Nucleotide Diversity								
			AB	AN	EL	EX	GI	HM	LI	NP	RI
350	solute carrier family 35 member B4 (slc35b4), mRNA, HSOK chromosome 6, UDP-xylose and UDP-N-acetylglucosamine transporter	GDP-frucose transmembrane transporter, carbohydrate transport, regulation of gluconeogensis, UDP-N-acetylglucosamine transport, UDP-xylose transport	—	—	—	0.455	0.420	—	0.494	0.480	0.469
458	exostosin-like glycosyltransferase 3 (extl3), mRNA	Axon guidance and regulation, chondroitin sulfate proteoglycan biosynthetic process, embryonic pectoral fin morphogenesis, heparan sulfate proteoglycan biosynthetic process, positive regulation of fibroblast growth factor receptor signaling pathway,	0.219	—	0.231	0.180	0.278	0.133	0.180	0.153	0.180

4033	coiled-coil domain-containing protein 63-like mRNA, Hd-rR chromosome 14 sequence, strain Hd-rR, complete genome assembly, chromosome 14	Cell membrane function	—	0.305	0.293	0.165	0.320	0.239	0.198	0.153	0.219
5902	RNA-binding protein MEX3B (LOC104929649), mRNA	RNA-binding	0.469	0.494	—	0.494	0.420	—	—	—	0.420
13729	RNA guanylyltransferase and 5'-phosphatase (rngtt), transcript variant X2, mRNA, mRNA-capping enzyme-like (LOC102797251), mRNA	GTP binding, mRNA guanylyltransferase activity, protein phosphotase activity, triphosphotase activity	0.305	0.117	0.219	0.236	0.180	0.219	0.320	0.320	0.219
14566	family with sequence similarity 129 member A (fam129a), mRNA, protein Niban-like (LOC109999805), mRNA, strain HSOK chromosome 4	Regulation of protein phosphorylation, response to endoplasmic reticulum stress	0.469	—	—	0.480	0.420	0.420	0.469	0.420	0.375
14987	large (Drosophila) homolog-associated protein 5 (dlgap5), transcript variant X2, mRNA	Protein assembly, signalling	0.500	0.500	0.490	0.463	0.500	0.469	0.495	0.500	0.469

15688	copine-8 transcript variant, mRNA	Possible calcium-dependent membrane-binding protein	0.255	0.278	0.305	0.320	0.278	0.172	0.180	0.180	0.219
16572	cadherin-related family member 1-like transcript variant, mRNA	Cell adhesion	—	0.346	0.293	0.397	0.444	0.399	0.346	0.320	0.375
17242	nucleoporin 98 transcript variant, mRNA	Nuclear core complex assembly	0.198	0.219	0.219	0.165	0.180	0.133	0.095	0.320	0.219
18034	nesprin-2-like (LOC109988425), mRNA	Actin filament binding	—	—	0.426	0.397	0.444	0.360	0.401	0.420	—
19666	neutral ceramidase-like (LOC108892356), mRNA, N-acylsphingosine amidohydrolase 2 (asah2), transcript variant X3, mRNA	Ceramide metabolic processes, multicellular organism development	—	0.278	0.180	0.165	0.320	0.210	0.198	0.278	—
19897	contig00136 genomic sequence, contig00151 genomic sequence	~	—	0.401	0.311	0.320	—	0.399	0.420	0.420	0.420
20385	major facilitator superfamily domain-containing protein 8-like (LOC109636640), mRNA, uncharacterized LOC104927507 (LOC104927507), mRNA	Transmembrane transport	—	0.219	0.133	0.236	—	0.245	0.095	0.153	0.219

20427	strain Hd-rR chromosome 22 sequence	~	0.278	0.198	0.305	0.236	0.320	0.172	0.320	0.320	0.320
21724	prostaglandin E synthase 2 (ptges2), mRNA, clone CFW204-C11 mRNA sequence	prostaglandin biosynthesis, lipid metabolism, enzyme regulation	—	0.346	0.391		0.420	0.387	0.278	0.320	0.375
22878	chromosome sequence corresponding to linkage group 1, top part, complete sequence	~	—	—	0.473	0.495	—	—	0.444	0.420	0.480
24552	pleckstrin homology and RhoGEF domain containing G4B (plekhg4b), transcript variant X3, mRNA	Rho guanyl-nucleotide exchange factor activity	0.444	—	0.473	0.434	—	0.459	0.444	—	0.469
24909	strain HSOK chromosome 14, MAP/microtubule affinity-regulating kinase 4-like (LOC110958165), transcript variant X2, mRNA	ATP-binding, protein serine/threonine kinase activity	—	0.401	0.477	0.463	0.480	0.482	0.475	0.480	—
30972	nibrin (nbn), transcript variant X2, mRNA	Damaged DNA-binding	—	—	0.500	0.500	0.500	—	0.492	0.500	—
32035	hippocampus abundant transcript 1 protein-like (LOC108897265), transcript variant X2, mRNA	Transmembrane transport	0.475	0.494	0.498	0.496	0.486	0.495	0.500	0.500	0.500

34598	histone-lysine N-methyltransferase SETD1B-A-like (LOC104931165), transcript variant X5, mRNA, SET domain containing 1B (Setd1b), mRNA	methyltransferase activity, RNA-binding, histone-lysine N-methyltransferase activity	—	—	0.293	0.180	—	0.219	0.255	—	0.219
34773	GTPase HRas-like (LOC109998395), mRNA, genome assembly common carp genome, scaffold: LG38, chromosome: 38	GTPase activity, GTP binding	—	0.305	0.293	0.351	0.278	0.349	0.320	0.320	0.420
36230	DnaJ heat shock protein family (Hsp40) member B5 (dnajb5), mRNA	Chaperone binding, unfolded protein binding	0.469	0.430	0.375	0.463	0.420	0.480	0.455	0.486	0.469
44669	transmembrane protein 236-like (LOC108883272), mRNA	~	—	—	—	0.180	0.180	0.229	0.346	0.180	0.219
44727	signal induced proliferation associated 1 like 3 (sipa1l3), mRNA	GTPase activator activity	0.198	0.346	0.219	0.298	0.320	0.210	0.278	0.278	0.320
46884	chromosome sequence corresponding to linkage group 1, top part, complete sequence	~	0.198	0.198	0.219	0.165	0.180	0.289	0.255	0.153	0.180

47816	solute carrier family 12 member 5 (slc12a5), transcript variant X2, mRNA, strain Hd-rR chromosome 5 sequence	Potassium chloride symporter activity, potassium ion symporter activity	—	0.500	0.497	0.484	0.480	0.496	0.500	0.500	0.500
50252	CUB and zona pellucida-like domain-containing protein 1 (LOC104939594), mRNA	Integral component membrane	0.255	0.278	0.231	0.236	0.153	0.278	0.278	0.278	0.180
51124	DnaJ heat shock protein family (Hsp40) member B5 (dnajb5), mRNA	Chaperone binding, unfolded protein binding	—	0.219	0.245	0.165	0.180	0.229	0.255	0.320	0.219
51751		Chaperone binding, unfolded protein binding	0.305	0.219	0.064	0.236	0.320	0.219	0.255	0.180	0.219
52092	growth/differentiation factor 11-like (LOC109141439), partial mRNA, neurogenic locus notch homolog protein 1-like, mRNA (LOC108875238,LOC106528756)	Metal binding, receptor activity	0.219	0.105	0.231	0.165	0.153	0.210	0.255	0.180	0.219
54277	tripartite motif-containing protein 16-like, mRNA (LOC106676446, LOC110001700, LOC109203666)	Zinc ion binding	0.475	0.475	0.391	0.434	0.420		0.475	0.480	0.375

55709	solute carrier family 43 member 3 (slc43a3), mRNA (LOC105932669)	Tansmembrane transport	0.430	—	—	0.463	0.420	0.484	—	0.480	—
58982	tetratricopeptide repeat protein 7A-like, partial mRNA (LOC104943395, LOC108895692), uncharacterized LOC103386891 (LOC103386891), transcript variant X2, ncRNA, tetratricopeptide repeat domain 7A (ttc7a), mRNA	Protein predicted	—	—	0.245	0.236	—	0.139	0.105	0.153	0.219
59223	ankyrin-1-like, transcript variant mRNA (LOC110963983,LOC104941581), ankyrin 1 (ank1), transcript variant X3, mRNA	Connects membrane proteins to cytoskeleton: cell motility, activation, proliferation	—	—	0.497	0.484	0.480	0.499	0.495	0.486	0.500
50345	chromosome sequence corresponding to linkage group 18, complete sequence		0.428	0.325	0.287	0.312	0.356	0.262	0.337	0.200	0.429

Supplementary Table 6.

Tag Location	Transmitter ID	Floy Tag ID	SL (cm)	TL (cm)	Weight (kg)	Weight (lb)	Sex
Hail Mary	13675	LI212	50.5	65.7	5.3	11.7	M
Hail Mary	10268	LI252	56	61	4.4	9.7	UK
Hail Mary	10264	LI253	62	70	6.37	14.0	UK
Hail Mary	10274	LI255	56	65	4.88	10.8	UK
Hail Mary	10272	LI257	50	60	3.8	8.4	UK
Hail Mary	10270	LI258	57	66	5.86	12.9	F
Hail Mary	10262	LI260	55	62	4.6	10.1	UK
Hail Mary	10271	LI262	53	63	3.26	7.2	UK
Hail Mary	10263	LI263	49	60	3.76	8.3	UK
Hail Mary	10277	LI264	54	62	3.3	7.3	UK
Hail Mary	10273	LI265	55	61	3.76	8.3	UK
Hail Mary	10275	LI266	57	66	4.43	9.8	UK
Hail Mary	10267	LI267	57	66	5.1	11.2	UK
Hail Mary	10265	LI268	56	65	5.46	12.0	UK
Hail Mary	10266	LI271	56	69	5.91	13.0	F
Hail Mary	10269	LI274	54	65	4.43	9.8	F
Hail Mary	10276	LI275	60	67	5.46	12.0	F
Hail Mary	15788	LI259	46	59.1	3.7	8.2	UK
Hail Mary	57265	LI201	54.3	69.3	6.6	14.6	UK
Hail Mary	57266	LI215	47.5	62.1	4.3	9.5	M
Hail Mary	57264	LI222	50.3	64.2	4.1	9.0	UK
Hail Mary	15791	LI226	45.1	57.1	3.3	7.3	UK
Hail Mary	15790	LI227	53.5	64.9	4.5	9.9	UK
Hail Mary	15789	LI272	47.5	58.8	3.9	8.6	UK
Hail Mary	57281	LI241	56.1	67	5.03	11.1	UK
Hail Mary	57275	LI232	55	63	4.26	9.4	UK
Hail Mary	57278	LI234	49	59.1	3.23	7.1	UK
Hail Mary	57277	LI254	57	70	5.44	12.0	UK
Hail Mary	57283	LI245	56	66.6	4.83	10.6	UK
Hail Mary	57279	LI224	56	66	5.23	11.5	UK
Hail Mary	57282	LI242	55.4	65.5	4.48	9.9	UK
Hail Mary	57284	LI228	61.5	72.9	7.19	15.9	UK
Hail Mary	57280	LI207	53	62.5	3.45	7.6	UK
Hail Mary	57269	LI118	57	68	5.54	12.2	UK
Hail Mary	57276	LI229	51	62	3.4	7.5	UK
Hail Mary	57270	LI105	51.2	63.3	4.44	9.8	UK
Hail Mary	57274	LI208	50	61	3.45	7.6	UK
Hail Mary	57267	LI239	45.9	55	2.39	5.3	UK
Hail Mary	57285	LI230	52	60.5	3.24	7.1	UK
Hail Mary	57273	LI225	41.2	53.1	2.45	5.4	UK
Hail Mary	57268	LI102	54.2	65.8	3.91	8.6	UK
Hail Mary	57272	LI233	51.5	61.5	4.11	9.1	UK
Hail Mary	57271	LI183	53.6	64	5.24	11.6	UK
Hail Mary	None	LI209	51.5	67.2	5	11.0	UK
Hail Mary	None	LI213	47	61.4	3.6	7.9	UK

Hail Mary	None	LI256	50	61	ND	ND	UK
Hail Mary	None	LI261	ND	60	ND	ND	UK
Hail Mary	None	LI270	53	55	ND	ND	UK
Hail Mary	None	LI244	36.5	44.1	1.4	3.1	UK
Hail Mary	None	LI250	28.5	35.2	0.9	2.0	UK
Hail Mary	None	LI236	27.5	33.6	0.52	1.1	UK
Hail Mary	None	LI210	30.9	38.5	0.7	1.5	UK
Hail Mary	None	LI249	25.4	31.5	0.47	1.0	UK
Hail Mary	None	LI206	26.9	33.9	0.69	1.5	UK
Long Island	57263	LI269	55	73	6.5	14.3	UK
Long Island	None	LI203	36.4	42.5	1.6	3.5	UK
Long Island	None	LI235	34.2	40.5	1.13	2.5	UK
Long Island	None	LI233	ND	ND	ND	ND	UK
Long Island	None	LI205	53	63.5	4.13	9.1	UK
Long Island	None	LI217	32	40	1.05	2.3	UK
Long Island	None	LI243	44.5	53.1	2.1	4.6	UK
Long Island	None	LI218	25	35.5	1.01	2.2	UK
Long Island	None	LI214	17.9	22.7	0.1	0.2	UK
Long Island	None	LI211	22.4	27.3	0.31	0.7	UK
Long Island	None	LI238	54.5	66.2	5.4	11.9	UK
Long Island	None	LI247	44.4	54.3	2.66	5.9	UK
Long Island	None	LI231	47.1	56.5	2.83	6.2	UK
Long Island	None	LI237	25	31.2	0.38	0.8	UK
Exuma*	None	EX116	51	65.8	5.3	11.7	UK
Exuma	None	EX184	40	46.2	1	2.2	UK
Exuma	None	EX187	26	34	0.7	1.5	UK
Exuma*	None	EX182	40	49.5	1.7	3.7	UK
Exuma	None	EX112	39.5	48	1.5	3.3	UK
Exuma	None	EX190	31	40.5	0.9	2.0	UK
Exuma*	None	EX0519	36.2	45	1.31	2.9	UK
Exuma	None	EX0510	33.3	42.1	0.9	2.0	UK
Exuma	None	EX0548	42	51.9	2.84	6.3	UK
Exuma	None	EX0511	34.1	41.8	1.23	2.7	UK
Exuma*	None	EX0532	28.4	35.1	0.74	1.6	UK
Exuma	None	EX0517	26	32.2	0.36	0.8	UK
Exuma	None	EX0504	29	36.2	0.68	1.5	UK
Exuma	None	EX0547	42	51.6	2.16	4.8	UK
Exuma*	None	EX0502	30.4	38.5	0.74	1.6	UK
Exuma*	None	EX0518	20	24.4	0.34	0.7	UK
Exuma*	None	EX0525	40.5	48.5	1.74	3.8	UK
Exuma*	None	EX0522	39.8	47.1	1.93	4.3	UK
Exuma	None	EX0508	34.2	40	1.1	2.4	UK
Exuma	None	EX0503	40.5	49.6	2.04	4.5	UK

Exuma	None	EX219	23.2	28.9	0.41	0.9	UK
Exuma	None	EX0513	23.5	29.6	0.35	0.8	UK
Exuma	None	EX0520	25	26	0.29	0.6	UK
Exuma	None	EX0524	26.5	31.5	0.5	1.1	UK
Exuma	None	EXJV1	17	21.2	0.08	0.2	UK
Exuma	None	EX204	33.3	40.5	1.05	2.3	UK
Exuma	None	EX216	19.7	24.4	0.24	0.5	UK
Exuma	None	EX220	21	35.4	0.73	1.6	UK
Exuma	None	EX0506	33.4	40.5	1.03	2.3	UK
Exuma*	None	EX0501	43.5	50.9	2.19	4.8	UK
Exuma*	None	EX0509	48	56	3.16	7.0	UK
Exuma*	None	EX0521	24	30.4	0.44	1.0	UK
Exuma*	None	EX0523	40.4	49.3	2.01	4.4	UK
Exuma	None	EX0527	37.9	46.2	None	ND	UK
Exuma	None	EX0526	47.4	58.4	3.16	7.0	UK

Chapter V

Stakeholder perspectives on the status and conservation management of Nassau grouper in The Bahamas

Manuscript in preparation

Authors: Sherman KD and Tyler CR

Stakeholders Perspectives on the Status and Conservation Management of Nassau grouper in The Bahamas

Krista D. Sherman[1,2,3*] and Charles R. Tyler*[1]

[1]Biosciences, University of Exeter, Stocker Road, Exeter, Devon EX4 4QD, UK

[2]Daniel P. Haerther Center for Conservation and Research, John G. Shedd Aquarium, 1200 South Lakeshore Drive, Chicago, IL 60605, USA

[3]Science and Policy, Bahamas National Trust (BNT), P.O. Box N-4105, Nassau, Bahamas

*Correspondence:
Krista Sherman
kds204@exeter.ac.uk
krista.daniellesherman@gmail.com

Professor Charles Tyler
C.R.Tyler@exeter.ac.uk

Abstract

Ensuring sustainability of marine resources that provide food, economic security and other critical ecosystem services is a major challenge globally. Assessing stakeholders' knowledge and perceptions of the status of fisheries and how they should be managed can yield important insights to facilitate the development of management frameworks that are likely to assist with achieving sustainability. As a socioeconomically and culturally important species, facing extinction, evaluating stakeholder perspectives on the status of the Nassau grouper fishery is critical to help inform policy. Knowledge and perceptions of stakeholders working in The Bahamas were assessed using voluntary participatory approaches including a workshop and questionnaires. As a pre-cursor to developing and disseminating questionnaires, a Strength Weakness Opportunities and Threats (SWOT) analysis was conducted with 15 individuals, revealing four common themes (centered around the environment, fisheries, legislation and enforcement and science, education and outreach) across stakeholders. Results from 26 questionnaires delved into these themes, and analysis of both qualitative and quantitative data highlighted similarities and differences between and within conservation and enforcement stakeholders. Perceptions about the status of Nassau grouper in various habitats, threats that impact the species, how the fishery should be managed, and the effectiveness of enforcement methods varied across stakeholder groups. However, stakeholders demonstrated strong support for science-based changes to current Bahamian fishery regulations along with the implementation of new regulations and increased capacity to better enforce and manage the fishery. The desire of stakeholders to support sustainable management for Nassau grouper was primarily based on concerns over ecosystem health, economic stability of the fishery, and the potential loss of the species.

Keywords: critically endangered species, fisheries management, socioeconomic drivers, marine policy, sustainability

Introduction

Anthropogenic activities have been instrumental in altering species and ecosystems (Foley et al. 2005; Jackson 2010; Cardinale et al. 2012). This finding is particularly profound for the marine environment and its associated fisheries, which supply 17 % of the protein required for human consumption worldwide, and up to 50 % of protein in Small Island Developing States, SIDS (FAO 2016). Yet, globally fisheries are under threat (e.g., Shahidul Islam and Tanaka 2004; Cheung et al. 2012), with recent reports estimating that >50 % of marine species have been overexploited (FAO 2016; Pauly and Zeller 2016, 2017). Thus, it is well-established that fisheries management is an important goal not only for food security, but also for the maintenance of biodiversity, ecosystem function and ecosystem services. However, while widely accepted, difficulties with marine resource management persist on national, regional and global scales, highlighting the need for interdisciplinary management approaches (Mora et al. 2009; Selkoe 2015; Wilson et al. 2016).

The Nassau grouper (*Epinephelus striatus*, Bloch 1792) represents one of the most highly valued commercial, subsistence and recreational fishery species in SIDS like The Bahamas (Cushion and Sullivan-Sealey 2008; Sadovy de Mitcheson and Colin 2012). The Bahamian Nassau grouper fishery remains lucrative, generating an average of more than US $1 million per year on average, but has declined significantly over the past 20 years (Sherman et al. 2016). In part, this has been attributed to decades of harvesting from fish spawning aggregations (FSAs), resulting in the decimation of Nassau grouper populations not only in The Bahamas (Smith 1972; Ray 2000; Stump et al. 2017), but throughout several countries in the Caribbean (Olsen and LaPlace 1979; Sala et al. 2001; Sadovy de Mitcheson et al. 2013). The respective listings of Nassau grouper as critically endangered and threatened on the IUCN Red List (Carpenter et al. 2015) and United States Endangered Species Act (Federal Register, 2016), further emphasise the urgency for more effective conservation management.

To this end, a range of management strategies has been implemented throughout the region in attempts to preserve remaining Nassau grouper populations and sustain the fishery. For example, fishing moratoriums have been implemented in the USA and Bermuda (Sadovy and Eklund 1999) and

temporary bans and seasonal closures in the Cayman Islands (Whaylen et al. 2007) and The Bahamas (Sherman et al. 2016). In 2015, the Bahamian government amended its fishery legislation to impose a permanent three month seasonal closure for Nassau grouper fishing during the spawning season. However, a multi-faceted approach is required to effectively manage the fishery because management strategies to date have not been effective in reversing declines of Bahamian Nassau grouper (Cheung et al. 2013; Sherman et al. 2016).

Numerous studies have shown the importance of assessing both biological and socioeconomic drivers to more effectively manage marine resources (Mora et al. 2009; Sadovy de Mitcheson and Erisman 2012; Hicks et al. 2016; Giglio et al. 2017). In a review of fisheries management practices for select fish species, Hilborn (2007) noted that successful fisheries were those that also assessed the behaviour of fishers and incorporated this information into policies. Consequently, more research has been undertaken to understand key drivers of fishers and other stakeholders (e.g. Yasué et al. 2010; Turner at al. 2014; Robinson et al. 2015). For example, temporal changes in abundances of goliath grouper (*Epinephelus itajara*), assessed using generational fisher knowledge, showed correlations between decreases in fish abundance with fishers' perspectives of best catches (Giglo et al. 2017). The authors noted than in addition to traditional management measures (e.g., fishing bans), diversification of sources of revenue would benefit the local Brazilian fishing community.

As a strategic planning tool, there are multiple uses for SWOT analyses, but most notably they are used to gather and synthesise information, which can be used to address a specified goal (Helms and Nixon 2010; Westhues et al. 2010). For example, Westhues et al. (2010) performed a SWOT analysis to evaluate the Canadian social education system and provided recommendations to address employee concerns. While difficult to execute, understanding and addressing social concerns can help to minimise conflicts and identify synergies between stakeholder groups, which can be used to develop mutually accepted management practices (Wilson et al. 2015). However, these approaches have not been implemented for conservation management efforts of Nassau grouper in The Bahamas. The purpose of the present research was to assess stakeholder knowledge regarding the status and management of the Bahamian

Nassau grouper fishery to aid the development of a national management plan for the species. Specific objectives were to 1) use SWOT analysis to identify important themes, potential barriers, and strategies to address threats and weaknesses viewed as relevant for managing Nassau grouper and 2) explore and describe similarities and differences in stakeholder knowledge and perspectives of Nassau grouper with respect to environmental change, fishery management, as well as science, education, and outreach initiatives in The Bahamas.

Methods

Two approaches were selected for stakeholder assessments: a workshop and questionnaires, as these were the most practical given the limitation on resources and time constraints. The workshop was held in January 2016 with 15 invited stakeholders including policy-makers, law enforcement officials, fishers, marine resource managers, scientists, non-governmental organizations (NGOs) and the private sector. All participants signed a consent form at the start of the workshop agreeing to have their responses recorded and analysed. Participants were divided into two groups to complete a Strength Weaknesses Opportunities and Threats (SWOT) analysis. The SWOT analysis was managed by facilitators (two per group) who were tasked with manually recording the outputs from group discussions (Supplementary Material 1). Each group attempted to address the following questions, which were also circulated via email one week prior to the workshop:

1. How would you characterize/describe (e.g. status, primary gears used, number of people involved, etc.) the Nassau grouper fishery in your island? in The Bahamas?
2. In your opinion what are the strengths/benefits of the Nassau grouper fishery?
3. How would your rank/prioritize these strengths/benefits?
4. In your opinion what are the threats/issues facing the Nassau grouper fishery?
5. How would you rank/prioritize these threats/issues?

6. Do you have any recommendations on how these threats/issues can be addressed?

Individual group responses were tabulated using Excel 2016 and similar or identical responses from both groups were collated and merged into a single table. Results from the SWOT analysis along with residual questions that were not addressed during the workshop helped to guide the development of comprehensive stakeholder questionnaires. Specifically, two semi-structured questionnaires were developed to assess stakeholder perspectives regarding the current status and potential management strategies for the Bahamian Nassau grouper fishery. The first questionnaire was designed for conservation stakeholders actively involved with marine resource management, environmental education/outreach, or advocacy in the country, and the second specifically for local enforcement officers. Both questionnaires contained closed and open questions to obtain quantitative and qualitative data. Open questions were designed to enable stakeholders to explain their opinions and provide any additional information perceived to be relevant for Nassau grouper management within The Bahamas. Electronic and hard copies of questionnaires were distributed to relevant government enforcement agencies (i.e. those legally tasked with marine resource management and enforcement) and marine protected area (MPA) wardens (enforcement stakeholders; n=55), and to local environmental NGOs, and fishery experts working in The Bahamas (conservation stakeholders; n=37). An overview of the research project along with objectives was provided at the beginning of each questionnaire. Participation was voluntary, stakeholders were permitted to omit questions, and they were informed that all responses would remain anonymous (Supplementary Materials 2-3). This approach was undertaken out of ethical responsibility and to elicit honest and direct feedback from participants. Additionally, all stakeholders completed a consent form to have their responses analysed prior to completing the questionnaire.

The first questionnaire, for conservation stakeholders actively involved with marine resource management, research, environmental education/outreach, or advocacy in the country, contained 48 questions and consisted of three sections: 1) Knowledge and Perceptions of Environmental Change, 2) Knowledge and Perceptions of Fisheries Management in The

Bahamas, and 3) Science, Education and Outreach. The second questionnaire, for local enforcement officers, consisted of 70 questions that asked the same questions in the conservation stakeholder questionnaire (sections 1-3) and additional questions pertaining to enforcing fishery regulations within The Bahamas (section 4: Legislation and Enforcement). To understand potential sources of variation in stakeholder responses, both questionnaires also contained an "About You" section, requesting demographic information including age, gender, nationality, educational history and employment (Supplementary Materials 2-3). Individual answers from respondents were coded (e.g. E1-E15 for enforcement and C1-C11 for conservation stakeholders) and manually entered into Excel prior to analysis.

In some instances, qualitative data has been represented in the form of anonymous (verbatim) quotes selected from various stakeholders. Descriptive statistics were used to analyse quantitative data. Venn diagrams were constructed using Venny v. 2.1 (Oliveros 2007-2015) by supplying lists of conservation and enforcement stakeholder responses, and other graphics (i.e. bar graphs, pie charts and tables) were also produced in Excel to visualise similarities and differences in stakeholder responses. Fishers did not form one of the stakeholder groups for the questionnaire because this approach was unlikely to be successful for a number of reasons (e.g., length of questionnaire, difficulties with technology/communication, lack of incentives). However, it was envisioned that key elements from these stakeholders could be used to develop a national survey comprised of a smaller subset of questions pertaining to the status and management of the Bahamian Nassau grouper fishery that would target a broader range of stakeholders including fishers and consumers. However, because all responses were not received until six months after the original deadline, there was insufficient time to develop, pilot test, and administer the national survey. Results from the SWOT analysis and returned questionnaires will be presented and discussed.

Results and Discussion

SWOT Analysis

Workshop participants identified 10 strengths, 18 weaknesses, 17 opportunities and 11 threats of the Nassau grouper fishery (Table 1). Results from the SWOT analysis helped to identify and organize common themes among stakeholders that were incorporated into the design of questionnaires to further explore perspectives related to: 1) environmental change, 2) fisheries management, 3) legislation and enforcement, and 4) science, education and outreach. Stakeholders were able to identify a number of natural and anthropogenic threats that Nassau grouper face. These threats have been linked to biological attributes of the species (e.g. delayed maturity, longevity and formation of FSAs) as well as unsustainable fishing, invasive alien species (IAS), inadequate fishery regulations and enforcement (reviewed by Sherman et al. 2016). However, the ecological, socio-economic and cultural values of the species are viewed as strengths and stakeholders were also able to identify more opportunities than threats, which can be applied to address weakness for the Bahamian Nassau grouper fishery (Table 1).

Table 1. SWOT analysis of the Bahamian Nassau grouper fishery. Stakeholder responses (verbatim) have been grouped into four themes: environment, fisheries, legislation and enforcement, science, education and outreach.

STRENGTHS	WEAKNESSES	OPPORTUNITIES	THREATS
Environment	**Environment**	**Environment**	**Environment**
We know that the species is threatened	Schooling behaviour for spawning makes them vulnerable	Need fishery-independent data to help management	Overfishing/Probably already below critical population threshold
Viable population in The Bahamas	Decreasing number/distribution of spawning schools	Conserve pelagic environment important for larvae	Low spawning stock biomass
	Unsustainable to fish spawning aggregations that are well known by fishermen and easy to harvest		Lionfish
			Potential climate change impacts (e.g. OA data deficient)
Fisheries	**Fisheries**	**Fisheries**	**Fisheries**
Economic benefits (direct & indirect, e.g. > $1 mil in revenue, trickle-down economics, higher valued compared to other fish species, etc.)	Need to amend closed season to include November & March due to early and late spawning	Data collection has improved	High demand by Bahamians and foreigners

Food source/diet (grouper bigger than other fish)	Boom fishery	Explore sustainable harvesting strategies for other species	No gear restrictions (e.g. hookah rigs, no trap limits)
	Monitoring of fishery	Collaboration among conservation partners to support development of sustainable fishery management plan	No bag limits/quotas
Cultural value/benefits			
	Not dealing with markets buying grouper but rather targeting fishermen	Aquaculture potential	Lack of alternative/sustainable livelihood options for fishermen
	Lack of support for fishermen (e.g. tax/duty free breaks)		
	Bureaucratic decision-making processes/Lack of governmental mechanisms to allow willing fishermen to participate in enforcement		
	Need Nassau grouper Fisheries Improvement Plan Working Group		
	Lack of sustainable fisheries plan for grouper		
Legislation and Enforcement	**Legislation and Enforcement**	**Legislation and Enforcement**	**Legislation and Enforcement**

Compliance by many Bahamians for closed season	Poor enforcement	New Fisheries Officers could enforce current regulations	Lack of compliance (e.g. illegal fishing during the closed season by foreigners and Bahamians and catching undersized fish)
More fisheries officers recently added to DMR	Family/community connection decreases willingness to enforce regulations	Training for Fisheries Officers (e.g. why regulations exist, what they are, conflict resolution, etc.)	Lack of enforcement
Fixed closed season		Increase fines for Bahamians and foreigners violating regulations	
		Change policy to allow ticketing/administrative fines	
		Fishermen willing to participate in collaborative patrolling/enforcement	
Science, Education and outreach	**Science, Education and outreach**	**Science, Education and outreach**	**Science, Education and outreach**
Public understands importance of species	Lack of education of fishermen	RBDF and Police could better support enforcement and research	Lack of knowledge/Misconceptions about biology/behaviour (e.g. Illegal fishing creates a "catch it while you can" mentality. Tragedy of the Commons)

Science/research is ongoing	Lack of knowledge of regulations by public Lack of funding for scientific research and FSA monitoring Source-sink dynamics unknown Educational/outreach materials not targeting right age groups	Communication strategy for changes to Fisheries Regulation 35 Some fishermen keen to get involved in research Intervene in markets (supply-demand chain)/outreach campaign designed for restaurants, hotels, fish processing plants, tourists, etc. More public awareness programs (e.g. local meetings, fishermen focus groups, workshops, walk-a-bouts, etc.) Develop material for recreational fishing and diving tourists	

Stakeholder demographics

A total of 26 (out of 92) respondents completed the questionnaire, corresponding to an overall response rate of 28 %. Response rates from conservation and enforcement stakeholders were (n=15; 41 %) and (n=11; 20 %) respectively. Additionally, as stakeholders were permitted to omit questions, answers were not provided for every question. Low response rates are common in social science studies, especially those that are not incentivised (Parfitt 2005; Singer and Couper 2008; Forster et al. 2011). However, due to the nature of the work undertaken by conservation and enforcement stakeholders, this option was not employed. Although the decision not to provide incentives may have negatively impacted response rates, we are likely to have received input from stakeholders that have a vested interest in the status of Nassau grouper in The Bahamas outside of their job responsibilities. Most respondents (73 %) were Bahamian and there was similar representation from both males (50 %) and females (42.3 %), although males dominated enforcement responses and females contributed more to conservation responses (Fig. 1). Stakeholders varied in age, with the majority of respondents ranging between 26-45 years (Fig. 1). Most respondents were well-educated, with conservation stakeholders possessing advanced qualifications up to the doctorate level (Fig. 1).

The frequency of Nassau grouper consumption by both stakeholder groups was low (Supplementary Fig. 1). In the 'other' category, two respondents stated they only consume Nassau grouper twice per year and one confirmed eating Nassau grouper three times per year (Supplementary Fig. 1). Taste (μ=8 $\pm$1.4 SD) followed by tradition/culture (μ=5 $\pm$1.4 SD) was the most common reason among stakeholders for consuming Nassau grouper (Supplementary Fig. 1). Many (46 %) stakeholders do not personally fish for the species (Supplementary Fig. 1). However, those that admitted to catching Nassau grouper (~7 % conservation and 18 % enforcement stakeholders) use traditional fishing techniques to harvest between 1-5 fish.

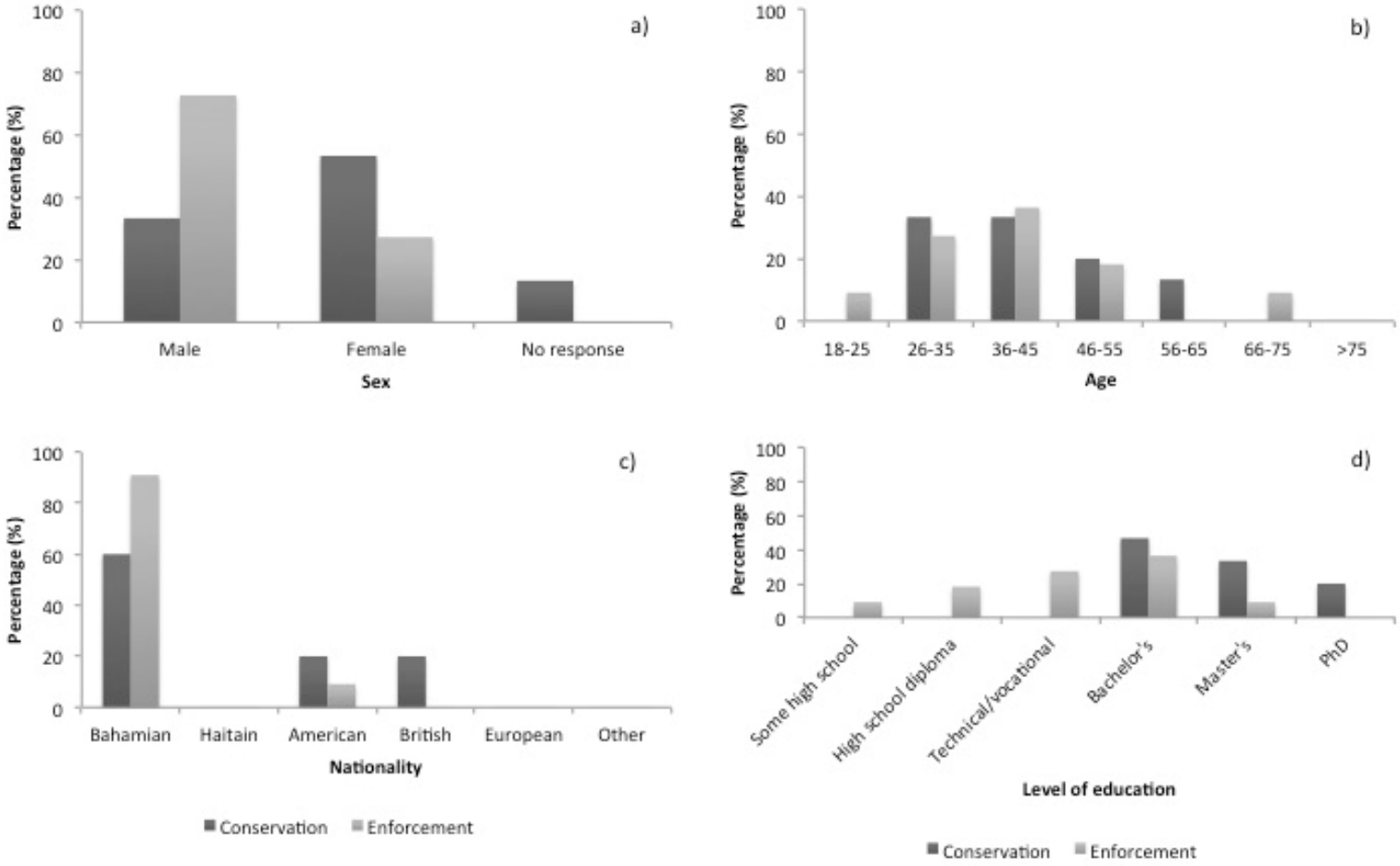


Figure 1. Demographic information from conservation and enforcement stakeholders including: a) sex, b) age, c) nationality and d) level of education.

With regards to the status of Nassau grouper on reef habitats, the majority of both stakeholder groups indicated that fish were in decline (Fig. 2). However, some enforcement stakeholders also believed that abundances of Nassau grouper have increased (27 %) or that there has been a change in abundance (9 %) on reefs (Fig. 2). Similarly, for nursery habitats, many stakeholders noted declines in Nassau grouper. However, 7 % of conservation stakeholders selected no change or increase in abundance, while 45 % of enforcement stakeholders indicated that Nassau grouper abundance has increased in nursery habitats (Fig. 2). With respect to trends in Nassau grouper landings over the past decade, stakeholder responses were generally similar, but more enforcement stakeholders indicated that landings had increased (Fig. 2). Stakeholders acquired information regarding the status of Nassau grouper from a variety of sources including personal observations (Fig. 2). However, scientific journals were used more by conservationists than enforcement officers (Fig. 2), which is unsurprising. The use of scientific journals and personal observations as an information source was also reflected in responses to question five: Please list the top 3-5 reasons why you believe there has been a decline/increase in Nassau grouper in The Bahamas (Table 2). Approximately, 4 % of enforcement stakeholders believed that the closed season has led to increases in Nassau grouper. Both stakeholder groups provided similar explanations for declines in abundance of Nassau grouper, but the top five reasons differed among conservationists and enforcement officers (Table 2; Fig. 3).

However, stakeholder perceptions regarding the status of Nassau grouper do not align with available scientific data, which show not only clear declines in landings (Sherman et al. 2016), but also declines in density throughout The Bahamas (Dahlgren et al. 2016; Sherman et al. In Press). These declines have been evidenced from fishery-independent data (i.e. habitat assessments) and fishery-dependent data (i.e. from commercial landings) and can be partially attributed to FSA fishing, which is also responsible for the collapse of several spawning sites in the country, e.g. Cat Cay, Bimini, High Cay, Andros, and others around Long Island (Sherman et al. 2016; Stump et al. 2017; Dahlgren et al. unpubl. data). Additionally, the legal removal of subadult

fish and illegal harvest of juveniles are also contributing to population declines within the country (Sherman et al. 2018). The use of personal observations as a source of information has likely contributed to stakeholder misconceptions regarding the status of Nassau grouper populations within the country. Previous studies have demonstrated that social concerns and stakeholder perspectives are important considerations for strengthening governance frameworks (Yasué et al. 2010; Turner et al. 2014, 2016) and shaping policy regulations to conserve species and their ecosystems (Hicks et al. 2016). Acknowledging and addressing stakeholder misconceptions should be prioritised as part of future management effectiveness strategies for Nassau grouper in The Bahamas.

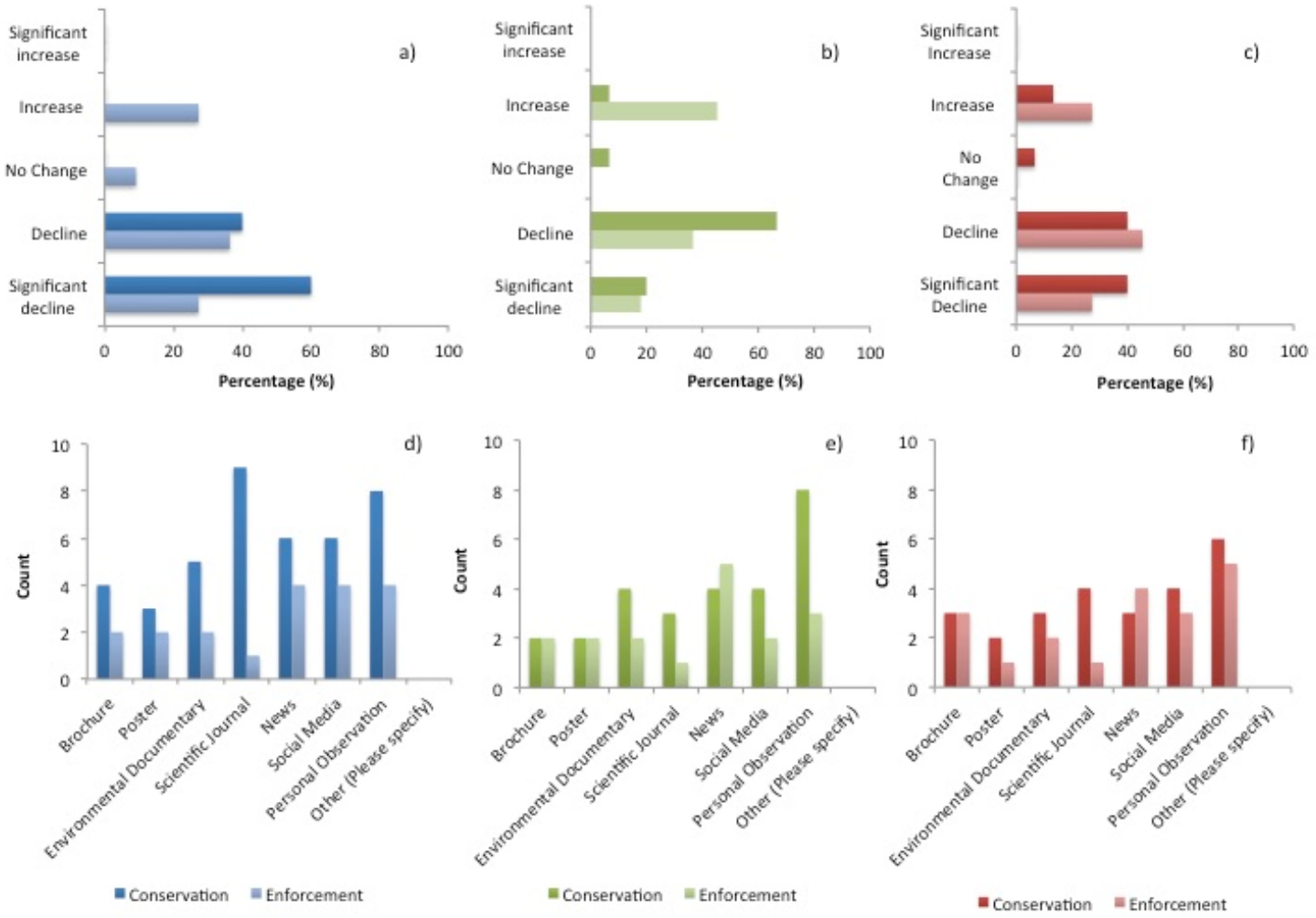

a)
Significant increase
Increase
No Change
Decline
Significant decline
0
20
40
60
80
100
Percentage (%)
b)
Significant increase
Increase
No Change
Decline
Significant decline
0
20
40
60
80
100
Percentage (%)
c)
Significant Increase
Increase
No Change
Decline
Significant Decline
0
20
40
60
80
100
Percentage (%)
d)
Count
10
8
6
4
2
0
Brochure
Poster
Environmental Documentary
Scientific Journal
News
Social Media
Personal Observation
Other (Please specify)
Conservation
Enforcement
e)
Count
10
8
6
4
2
0
Brochure
Poster
Environmental Documentary
Scientific Journal
News
Social Media
Personal Observation
Other (Please specify)
Conservation
Enforcement
f)
Count
10
8
6
4
2
0
Brochure
Poster
Environmental Documentary
Scientific Journal
News
Social Media
Personal Observation
Other (Please specify)
Conservation
Enforcement

Figure 2. Results of stakeholder perspectives of the status of Nassau grouper for a) reefs (blue), b) nursery habitats (green), and c) landings (red) and where they obtained this information from d-f) for the corresponding habitats (reefs and nurseries) and landings.

Table 2. Five common reasons among stakeholders for increases/decreases in Nassau grouper.

	Conservation Stakeholders	Enforcement Stakeholders
Ranking	**Top reasons for increase/decrease in Nassau grouper**	**Top reasons for increase/decrease in Nassau grouper**
1	Poor regulations (10 %)	Illegal fishing (10.7 %)
2	Gleaned from scientific information (8.9 %)	Overfishing (8 %)
3	FSA fishing (6.7 %)	Closed season (4 %)
4	Illegal fishing (5.6 %)	Fishing undersized Nassau grouper (4 %)
5	Unsustainable fishing practices (5.6 %)	FSA fishing (2.7 %)

Table 3. Prioritised top five threats to Nassau grouper in The Bahamas.

	Conservation Stakeholders	Enforcement Stakeholders
Ranking	**Top threats to Nassau grouper**	**Top threats to Nassau grouper**
1	Habitat degradation/destruction (11.6 %)	Illegal fishing (21.7 %)
2	FSA fishing (10.5 %)	Overfishing (8.3 %)
3	Lack of enforcement (6.3 %)	Habitat degradation/destruction (6.7 %)
4	Overfishing (5.3 %)	FSA fishing (3.3 %)
5	Fishing undersized Nassau grouper (5.3 %)	Fishing undersized Nassau grouper (3.3 %)

Table 4. Stakeholder rankings of the main reasons to conserve or not conserve Nassau grouper.

	Conservation Stakeholders	Enforcement Stakeholders
Ranking	**Top reasons to conserve or not conserve Nassau grouper**	**Top reasons to conserve or not conserve Nassau grouper**
1	Ecosystem health (23.6 %)	Species survival (17.5 %)
2	Economically important (16.4 %)	Ecosystem health (17.5 %)
3	Culturally important (16.4 %)	Food source/taste (10 %)
4	Species survival (10.9 %)	Sustain fishery (7.5 %)
5	Food source/taste (9.1 %)	Economically important (7.5 %)

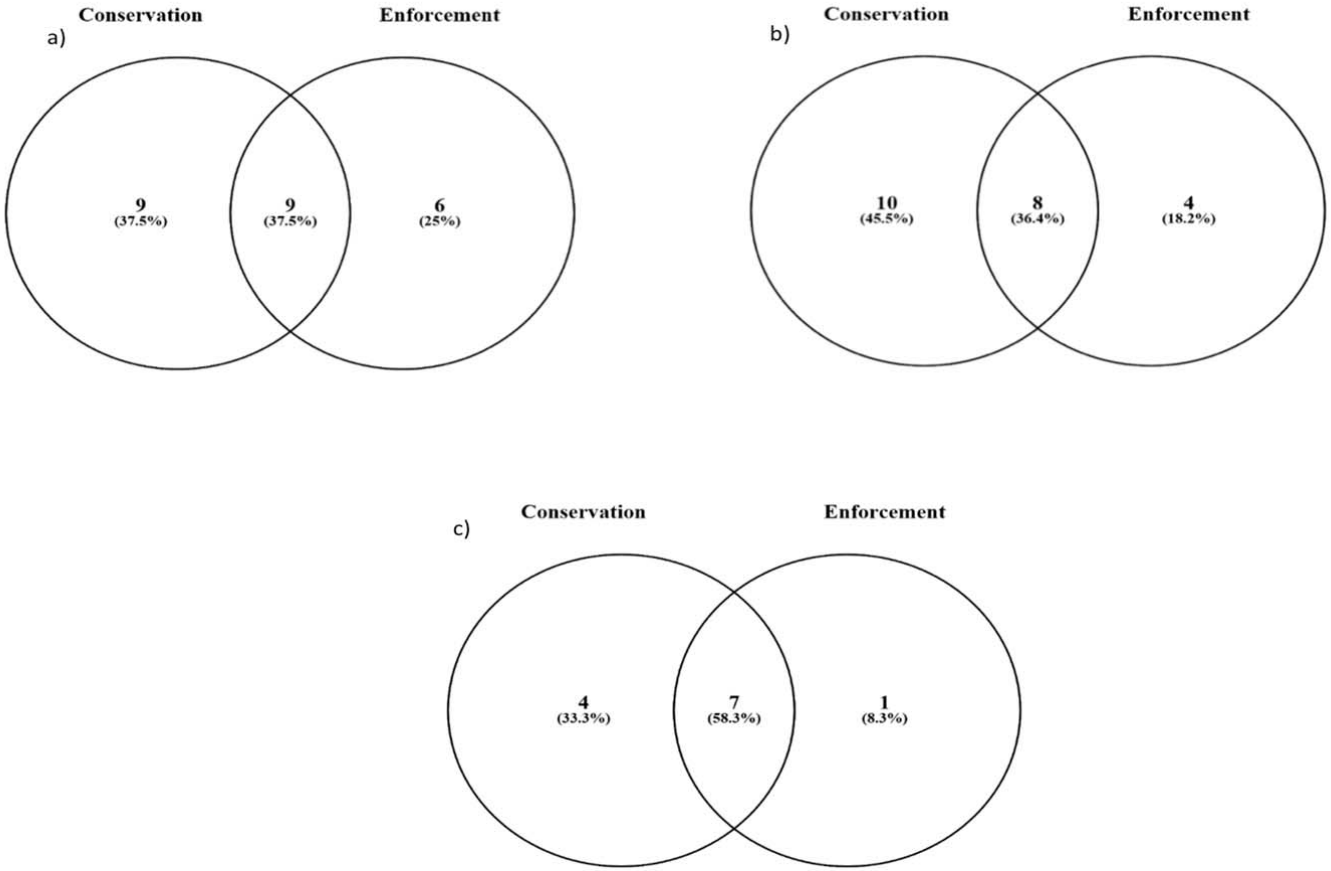

a)
Conservation
Enforcement
9
(37.5%)
9
(37.5%)
6
(25%)
b)
Conservation
Enforcement
10
(45.5%)
8
(36.4%)
4
(18.2%)
c)
Conservation
Enforcement
4
(33.3%)
7
(58.3%)
1
(8.3%)

Figure 3. Venn diagram illustrating similarities and differences in conservation and enforcement stakeholder's views on a) reasons for increases/decreases in Nassau grouper, b) top five threats to Nassau grouper and c) reasons to conserve/not conserve Nassau grouper.

There were four common threats among stakeholder groups, but these differed in order of importance (Table 3; Fig. 3). Conservation stakeholders ranked habitat destruction/degradation as the most important threat to Nassau grouper. In comparison, enforcement stakeholders listed illegal fishing as the most important threat to the species (Table 3; Fig. 3). For enforcement stakeholders, illegal fishing was also the principal reason for declines of Nassau grouper (Table 2). Both stakeholder groups expressed concern for the sustainability of Nassau grouper (Supplementary Fig. 2). However, this question was omitted by 46.7 % of conservation stakeholders. When asked why it was important or not to conserve Nassau grouper, three out of five responses were similar between stakeholders, with ecosystem health, economic importance and survival of the species emerging at the top of the list (Table 4; Fig. 3).

Knowledge and Perceptions of Fisheries Management

More than 80 % of stakeholders were able to correctly identify the dates for the closed season and the minimum size limit for Nassau grouper (Fig. 4). However, both stakeholder groups provided incorrect responses, which gives cause for concern as these stakeholders are responsible for not only managing the resource, but also educating the public on fishery regulations and the status of the species. Failure of these groups to answers these questions with 100% accuracy highlights the need for better transmission of information and training. Stakeholders displayed varying responses regarding the effectiveness of different methods for managing Nassau grouper (Fig. 5). Conservation stakeholders mostly viewed the minimum size limit as ineffective, whereas more enforcement stakeholders thought this regulation was neither effective or ineffective or very effective (Fig. 5). Conflicting responses within the enforcement group, appear to be based on their understanding of the benefits of the regulation, perceived benefits/personal observations of benefits of the regulation or how well the regulation has been enforced. For example, stakeholders provided the following explanations for their responses:

"Theoretically gives groupers a chance to spawn at least once. Needs enforcement". — Enforcement stakeholder

"Seeing more juveniles". — Enforcement stakeholder

“Not adhered to”. — Enforcement stakeholder

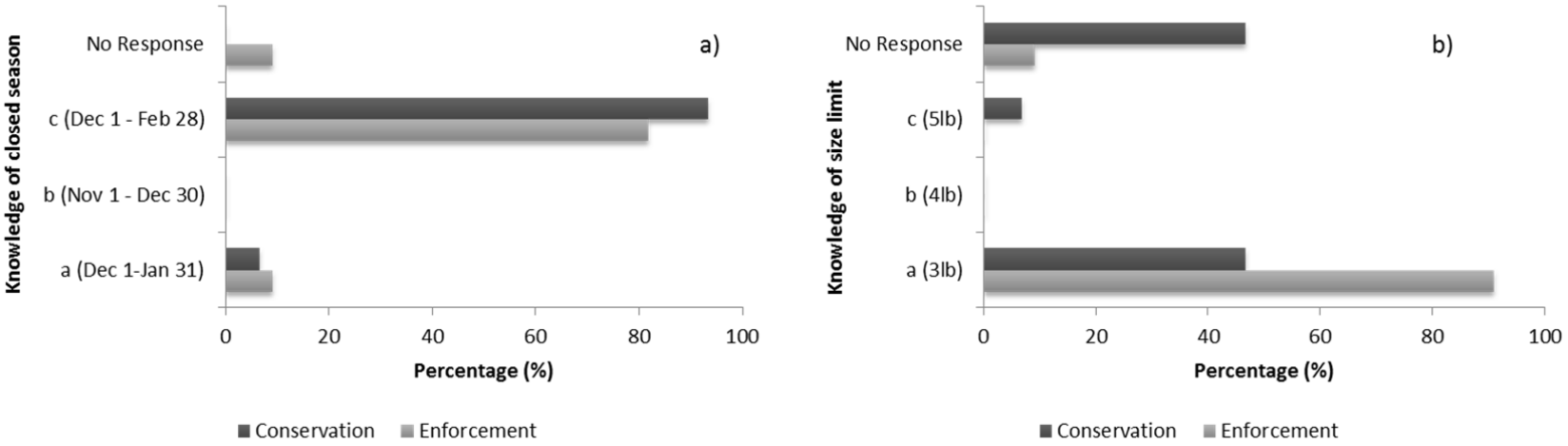


Figure 4. Results of stakeholder knowledge of a) the Nassau grouper closed season and b) current size limits within The Bahamas.

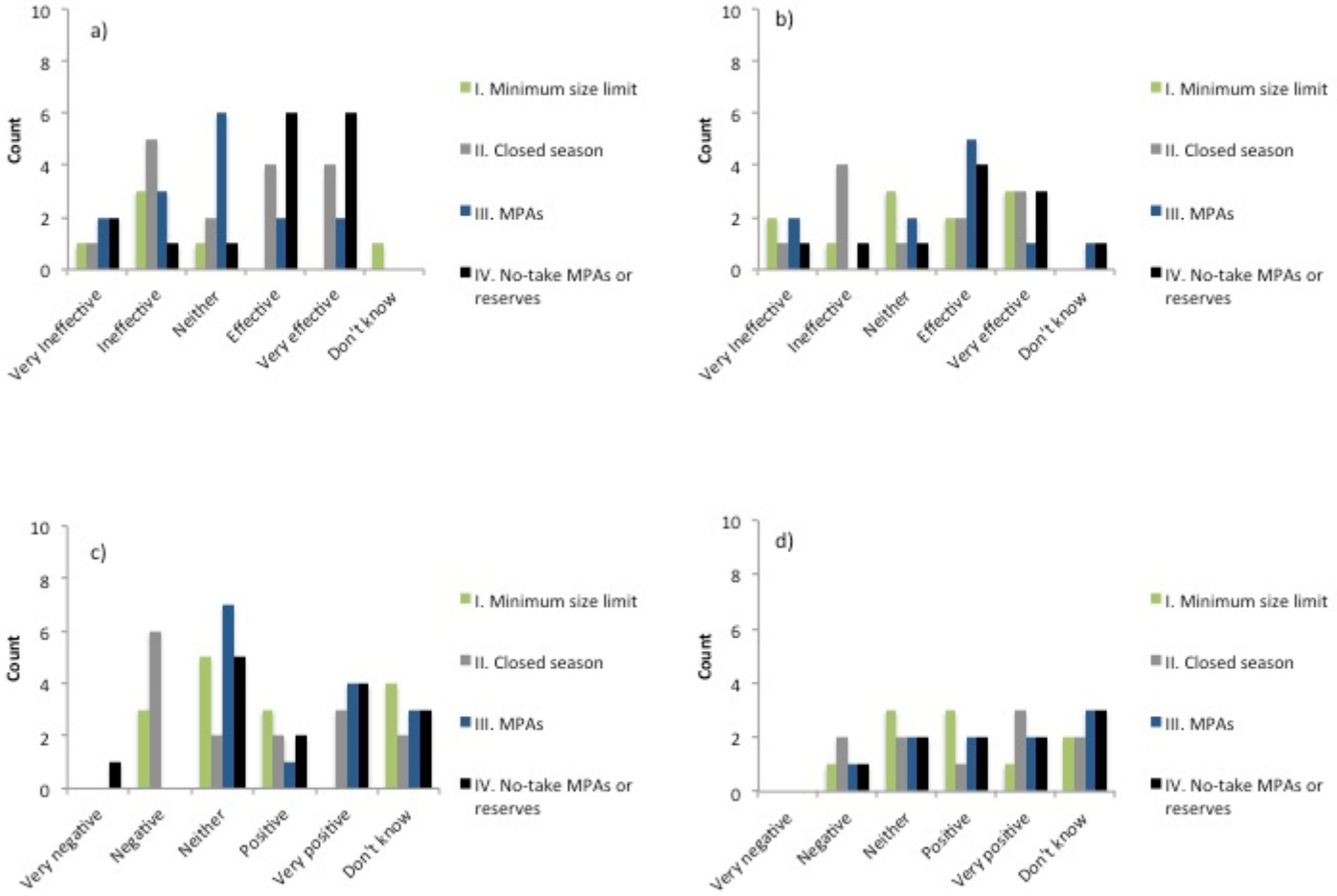


Figure 5. Conservation and enforcement stakeholder perspectives on management effectiveness (5a and 5b) for Nassau grouper and how this affects Bahamian fishers (5c and 5d) with respect to the minimum size limit (green), closed season (grey), MPAs (blue), and no-take MPAs or reserves (black).

A range of opinions was expressed regarding the effectiveness of the closed season, with slightly more responses in favour of it being ineffective (five and four for conservation and enforcement stakeholders respectively (Fig. 5). No-take MPAs were considered to be more effective than MPAs, but interestingly, 40 % of conservation stakeholders viewed both methods as neither effective or ineffective (Fig. 5). When asked to reflect on how fishery policies for Nassau grouper may impact Bahamian fishers, conservation stakeholders perceived them to be neither positive nor negative whilst enforcement stakeholders had varying opinions (Fig. 5; Box 1).

Box 1. How do Nassau grouper fisheries regulations affect Bahamian fishermen?

I. Minimum Size Limit

"No impact". "Don't know". — *Conservation stakeholder*

"I would hope the current management strategies would improve fishermen's livelihoods in the long run". — *Conservation stakeholder*

"No real affect because most grouper fishers target the larger fish, for more money". — *Enforcement stakeholder*

"Fishers pay little attention to minimum size". — *Enforcement stakeholder*

II. Closed season

"Short term this is negatively affecting fishermen but will be positive if we can prevent the aggregations from disappearing". — *Conservation stakeholder*

"The closed season hasn't been in place long enough to force fishers to adjust their harvesting". — *Conservation stakeholder*

"I don't think there is an affect on the fishermen at this time because we have been having this seasonal closure for 18 years and fishermen have adapted". — *Enforcement stakeholder*

"Most obey the closed season, but there are still those who fish on the spawns and continue to take grouper". — *Enforcement stakeholder*

III. Marine protected areas (MPAs)

"Helps support healthy reefs and in return multiplies fisheries". — *Conservation stakeholder*

"Presently they are not managed effectively, has no benefit or cost". — *Conservation stakeholder*

"Too early to tell". — *Enforcement stakeholder*

"Allows fish to reproduce and venture into other areas so fishermen would have fish to catch". — *Enforcement stakeholder*

IV. No-take MPAs or reserves

"Dependent on the design and time line – if replenishment if the goal it takes a long time and some fishermen argue they are the loser in the scenario". — *Conservation stakeholder*

"Only tool proven effective in Bahamas to protect spawning stock and support fishery". — *Conservation stakeholder*

"MPAs and no-take MPAs prevent fishermen from harvesting groupers. The effect is felt through less financial gain". — *Enforcement stakeholder*

"These areas are not harvested but there is a spill over effect". — *Enforcement stakeholder*

Stakeholders were in favour of supporting changes to existing regulations and new regulations for Nassau grouper (Fig. 6). Proposed amendments to existing regulations received similar responses, although more conservation stakeholders voted for increasing the minimum size limit (87%) and establishing new no-take MPAs (93 %) (Fig. 6). This finding was interesting, however, because conservation stakeholders viewed MPAs as neither effective or ineffective (Fig. 5), yet have also advocated for the expansion of the marine protected area system in The Bahamas (Knowles et al. 2017). This implies that although conservation stakeholders may have concerns regarding MPA effectiveness within The Bahamas, MPAs are still considered to be valuable method for resource conservation. Stakeholder sentiments provide further support for this observation:

"MPAs do not work because hardly "managed" if at all". — Conservation stakeholder

"Capacity to manage and protect MPAs is limited but should still be declared". — Conservation stakeholder

"Best method with proper enforcement". — Conservation stakeholder

Additionally, previous studies have demonstrated that personal experiences, social, economic and other factors help to shape stakeholder attitudes and perceptions (Innes 1998; Saenz-Arroyo et al. 2005; Gringart et al. 2008).

Protection of FSAs was the most common response provided by both stakeholder groups for new regulations (Fig. 6). Conversely, implementing a temporary ban received the least support from both stakeholders (Fig. 6). An 8-yr temporary ban on harvesting Nassau grouper during the spawning season was implemented in the Cayman Islands in 2003 following precipitous declines in spawning stock abundance (Whaylen et al. 2004) and was subsequently extended for eight more years in 2011 by the Cayman Islands Marine Conservation Board. FSA monitoring data indicates that this strategy has been successful in helping Cayman populations of Nassau grouper to recover (Whaylen et al. 2007; Heppell et al. 2012). Limited support for a temporary ban from stakeholders actively involved with Nassau grouper management, however, may be an indication that consumers, fishers and other stakeholders would also exhibit limited support for this management measure.

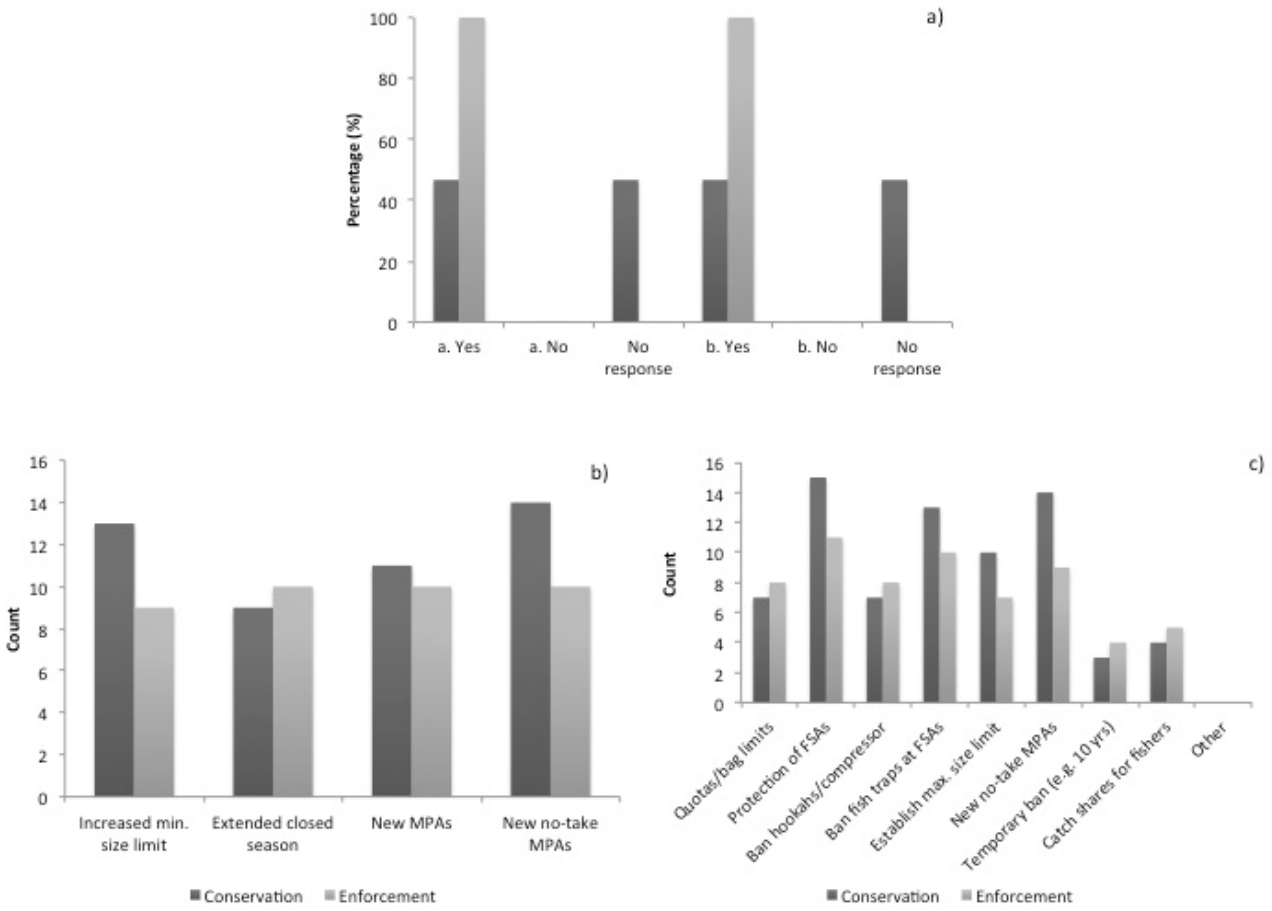


Figure 6. Results of stakeholder responses for a) willingness to support changes to fishery regulations b) support for changes to existing regulations and c) support for changes to new fishery regulations.

Across both stakeholder groups, consumer demand (μ=9 $\pm$2.8 SD) was perceived to be the most important driver for illegal fishing in The Bahamas. However, this perception differed between groups. Conservation stakeholders ranked consumer demand as the most important driver, whereas enforcement stakeholders viewed foreign commercial fishing as the most important driver of illegal fishing during the closed season (Fig. 7). Socioeconomic drivers have been linked to declines in many species (Purcell and Pomeroy 2015; Pomeroy et al. 2016) and can also impact desired management outcomes (Levin et al. 2017). Indeed, economic incentives have been shown to be key drivers for exploiting FSAs (Sadovy de Mitcheson and Erisman 2012; Robinson and Samoilys 2013). Contextual differences between these stakeholder groups are plausible explanations for their selection of different drivers. For example, enforcement stakeholder perspectives are likely to be reflective of their experiences addressing illegal fishing activity, whereas conservation stakeholders are less likely to base their decisions on this type of experience. Nonetheless, illegal fishing (foreign and domestic) of Nassau grouper does occur in The Bahamas (The Bahamas Maritime Authority, 2017), but underpinning the key driver(s) of this practice will require additional research.

When asked how barriers to effective management could be addressed, several common responses emerged among stakeholders. Strengthening enforcement capacity through: 1) the allocation of sufficient resources for staff acquisition, training, and surveillance throughout the archipelago, 2) improved inter-organisation cooperation, and 3) supply-demand market interventions were some of the identified strategies for overcoming management challenges to adequately protect the species (Table 5).

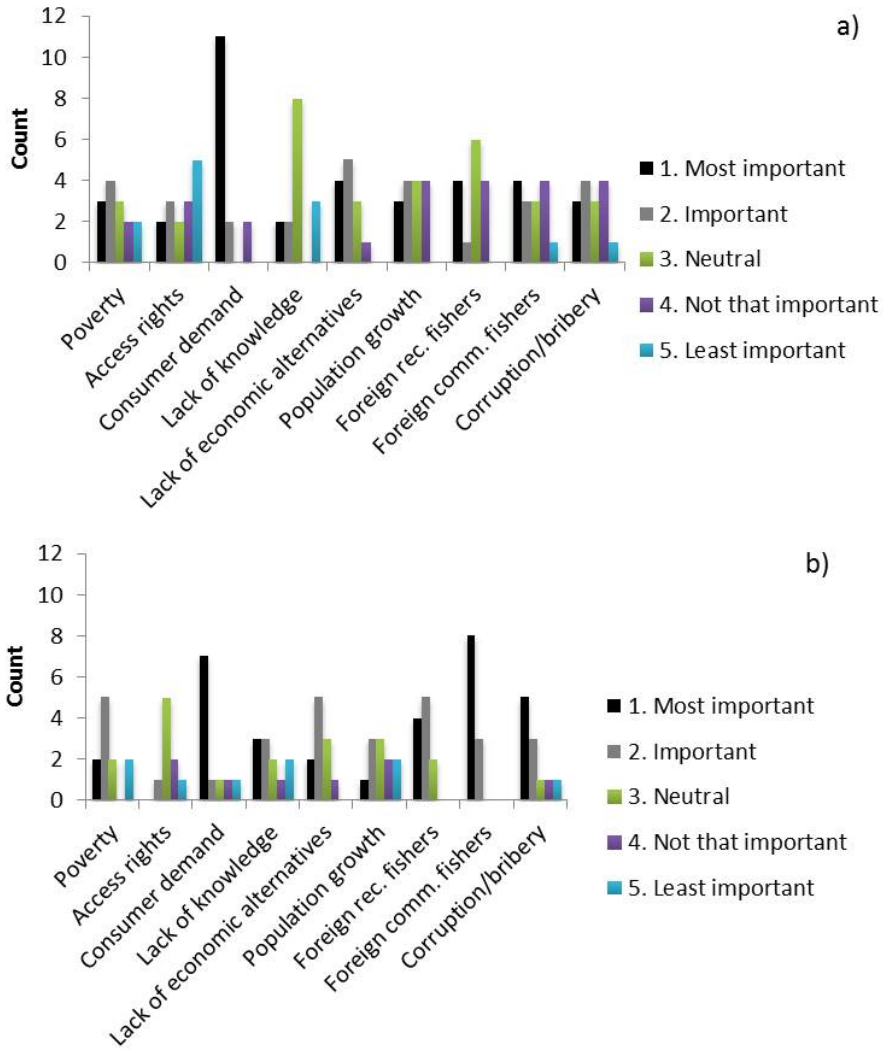


Figure 7. Conservation a) and enforcement b) stakeholder perceived drivers of illegal fishing for Nassau grouper in The Bahamas.

There was consensus amongst stakeholders with regards to the organisations involved in making management decisions for Nassau grouper (Supplementary Fig. 3). However, this pattern differed somewhat pertaining to organisations that should be managing the fishery (Supplementary Fig. 3), implying that perhaps there may be uncertainty over organisational responsibilities. Addressing this can help to improve efficiency among management authorities within the country.

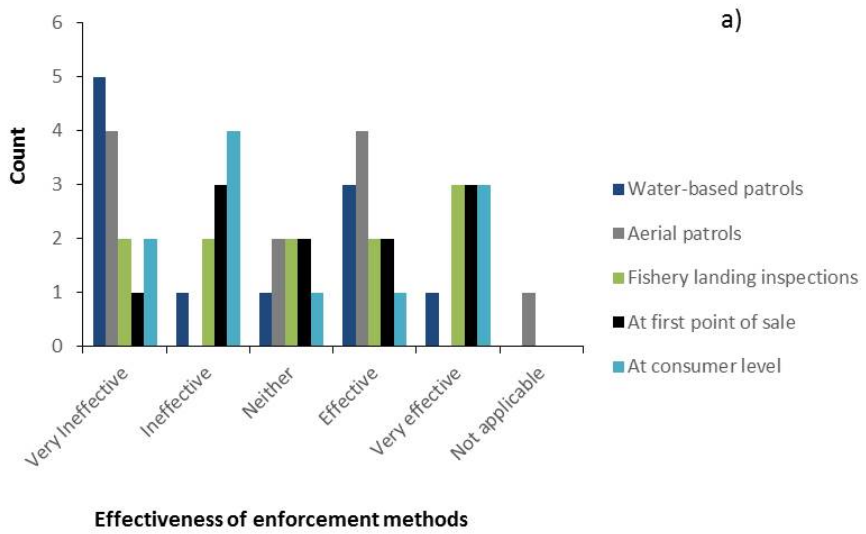
Legislation and Enforcement

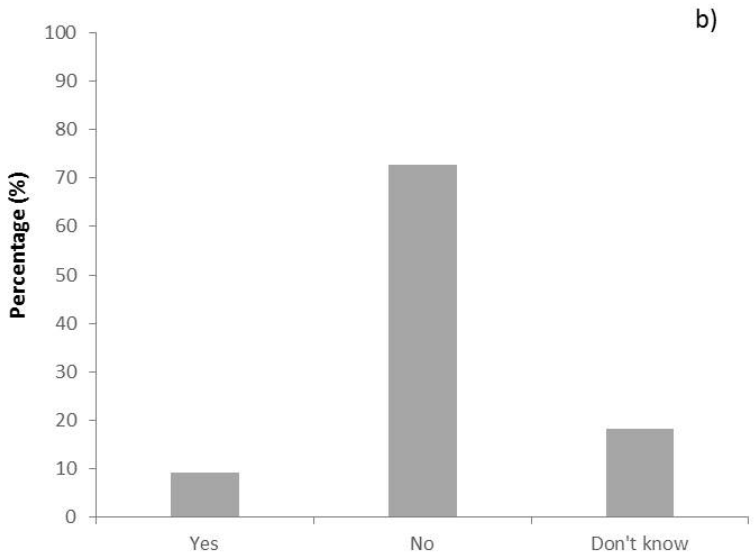
Enforcement stakeholders expressed contradictory opinions regarding the effectiveness of methods used to manage Nassau grouper. For example, water-based and aerial patrols were viewed as both very ineffective and effective (Fig. 8). Similarly, enforcement at the consumer level was also perceived as both ineffective and very effective. Enforcement stakeholders were limited in their ability to correctly identify all the agencies that are legally mandated to enforce fishery regulations (Supplementary Fig. 4). Organisations with legal responsibility for fishery regulations and enforcement in The Bahamas include the Department of Marine Resources (DMR), Royal Bahamas Defence Force (RBDF), Royal Bahamas Police Force (RBPF), Customs and Bahamas National Trust (BNT) via MPA wardens. For these stakeholders, RBDF and DMR were the top two selected organisations that should be enforcing fishery regulations (Supplementary Fig. 4).

A few enforcement officers were unaware of on-going training programs and the frequency at which they were offered. For example, one respondent indicated, “Not sure…..I know we (DMR) don’t”, while others included DMR on the list of organisations offering training. Thus, there appears to be some confusion among enforcement stakeholders regarding the training options that are currently available to them. This implies a breakdown in communication and/or a lack of consistency with training efforts and highlights a need for increased cooperation within and across enforcement agencies. However, there was considerable support for the development and implementation of enforcement training programmes, as 73 % of respondents believe that current resources are insufficient for effective enforcement. Training manuals and workshops/presentations were the top two types of requested training materials (Fig. 8). After collating and ranking responses, lack of resources/funding emerged as the primary difficulty encountered by enforcement stakeholders (Table 5). Varied approaches have been used in attempts to resolve these issues (Box 2), but it is unclear whether these have been successful.

Table 5. Ranked enforcement stakeholder perspectives of the main difficulties encountered when enforcing fishery regulations.

Ranking	Top difficulties with enforcement
1	Lack of resources/funding
2	Lack of training/trained staff
3	Lack of information/outdated information
4	Lack of support and corruption
5	Inadequate fees/penalties


a)
Count
6
5
4
3
2
1
0
Very Ineffective
Ineffective
Neither
Effective
Very effective
Not applicable
Effectiveness of enforcement methods
Water-based patrols
Aerial patrols
Fishery landing inspections
At first point of sale
At consumer level


b)
Percentage (%)
100
90
80
70
60
50
40
30
20
10
0
Yes
No
Don't know

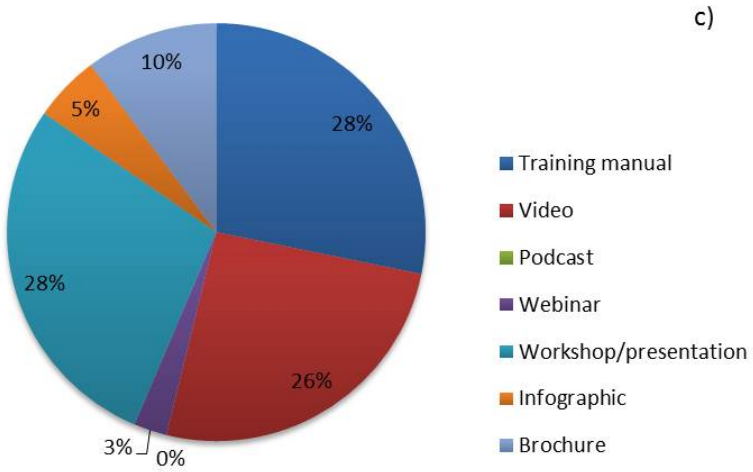

c)
10%
5%
28%
28%
26%
3%
0%
Training manual
Video
Podcast
Webinar
Workshop/presentation
Infographic
Brochure

Figure 8. Enforcement stakeholder perspectives regarding a) the effectiveness of enforcement methods, b), the adequacy of existing training programs and c) pie chart showing preferred training materials for officers.

Annual reports of illegal fishing activity along with associated fines/arrests were relatively low (1-5 was the most selected response) (Supplementary Fig. 5). However, enforcement officers were unable to confidently provide information on the number of arrests attributed to Bahamian versus foreign fishers (Supplementary Fig. 5). Several enforcement officers elected not to answer and others stated, …" not sure", "don't know", or "unknown" in response to questions 49 and 50 (Supplementary Material 2). Given the uncertainties surrounding this issue, in addition to the low sample size, the results presented should not be considered as representative for The Bahamas.

There was strong support by 91 % of respondents to increase penalties assigned to those who violate fishery regulations (Fig. 9). Currently, penalties for not adhering to Bahamian fishery regulations for Nassau grouper include a $5,000 fine and/or imprisonment for one year if found guilty (Fisheries Resources Jurisdiction and Conservation Act). In comparison, penalties for FSA fishing in the Cayman Islands are $500,000 or up to one year in prison (http://www.gov.ky/portal/pls/portal/docs/1/12326595.PDF). Establishing and enforcing penalties of this magnitude are likely to be more of a deterrent to illegal fishing and merit consideration for The Bahamas. However, it appears that clearer reporting mechanisms and maintenance of enforcement data are also needed. A well-maintained database would also allow for assessments of management effectiveness.

Box 2. Have there been any attempts to resolve these issues and if so what was the outcome?

"Yes, new ships; decentralized areas of operation". — *Enforcement stakeholder*

"No!" — *Enforcement stakeholder*

"Attempts are ongoing to solicit funding but the funds are slow coming. Our organization authority is also limited to national parks". — *Enforcement stakeholder*

"Outreach to fishing communities near MPAs has helped to explain value of regulations to longterm stability of industry. BNT looking to expand methods of revenue creation to increase staff and training programmes ". — *Enforcement stakeholder*

"Training has been carried out through partnerships with various enforcement agencies but more of these sessions are needed. Funding shortfalls are a major issue". — *Enforcement stakeholder*

"Not sure...please speak to Director of Marine Resources". — *Enforcement stakeholder*

"Yes, resulted in more fishing fines and catching of more violators ". — *Enforcement stakeholder*

"Working on it all the time". — *Enforcement stakeholder*

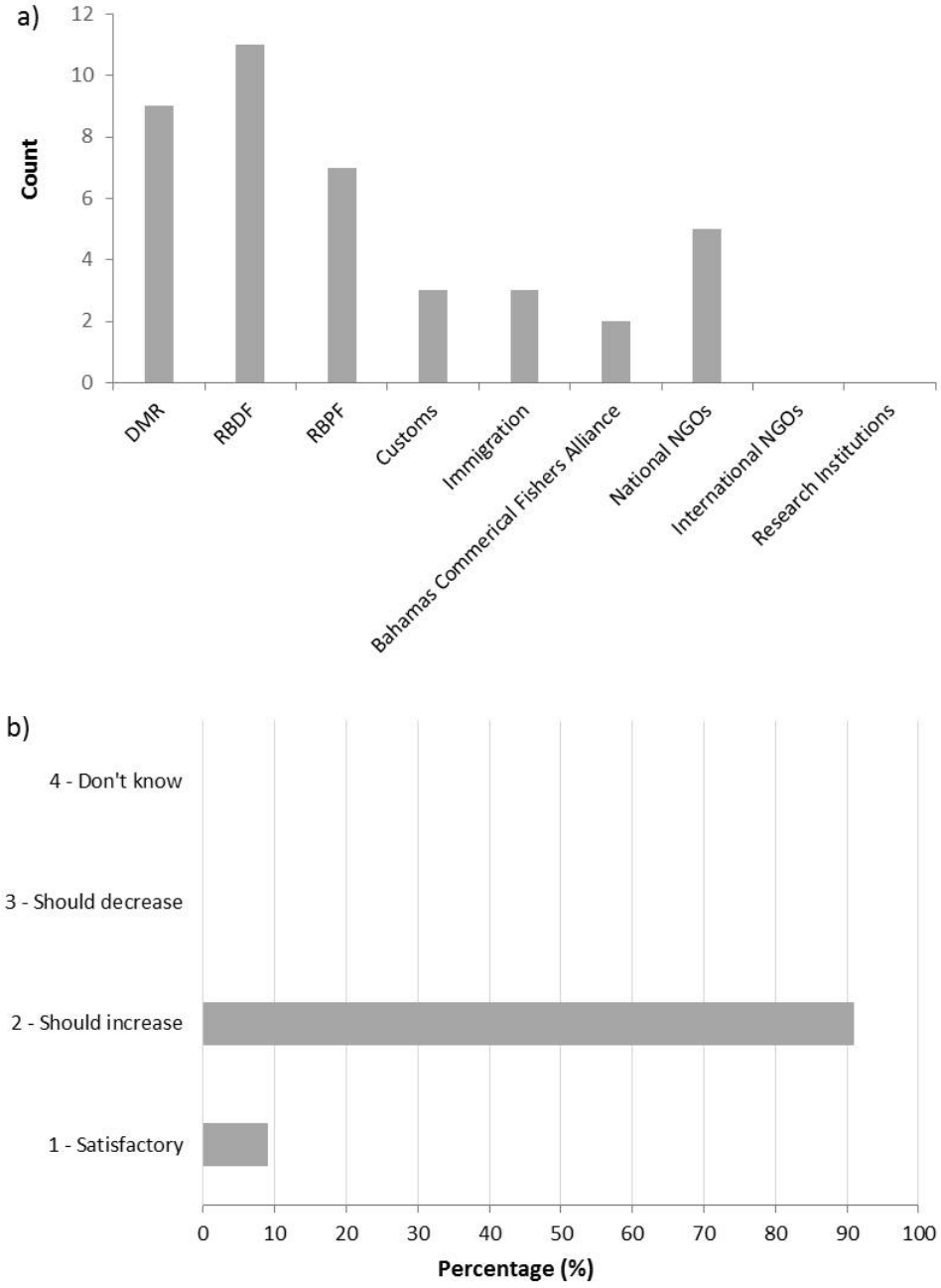
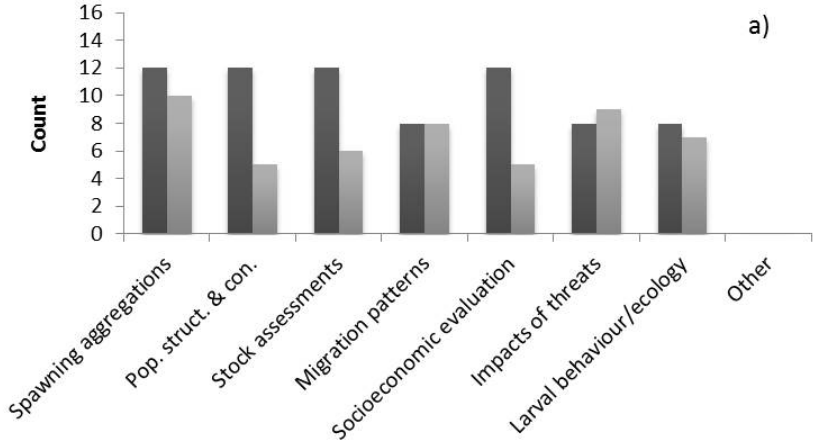
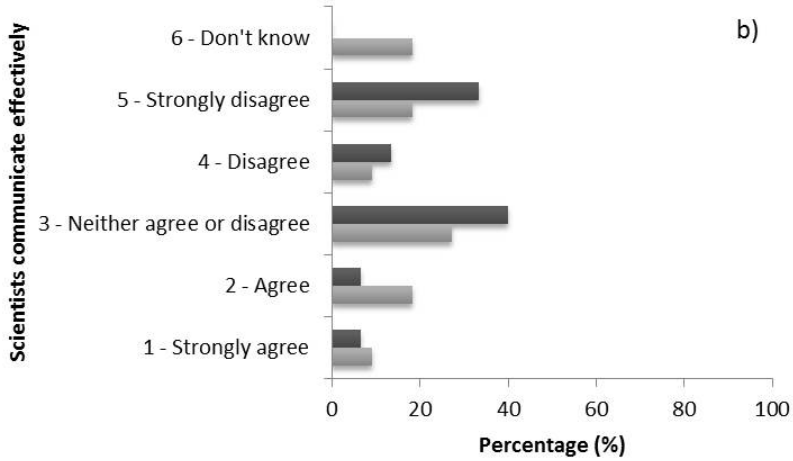
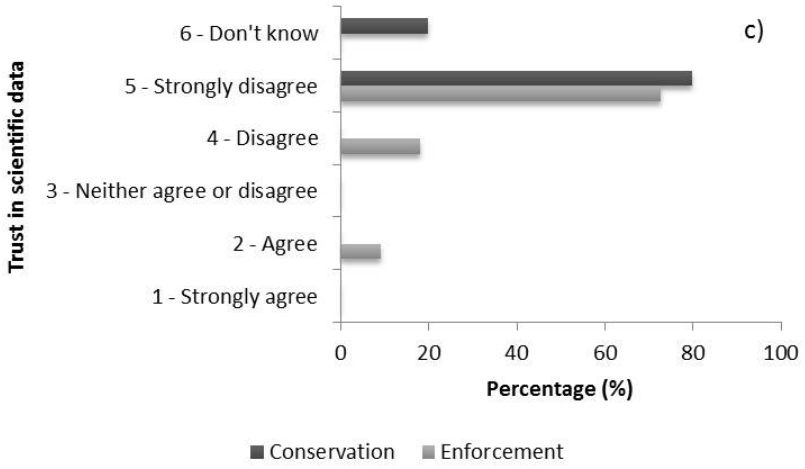


Figure 9. Support for increasing penalties for offenders of fishery regulations in The Bahamas.

Several research areas were perceived to be important by conservation stakeholders, including assessing spawning aggregations, population structure and connectivity, stock assessments and socioeconomic evaluations (Fig. 10). However, combined, both stakeholder groups (35 %) selected “Don’t know” when asked to comment on the usefulness of genetics to assess numbers of Nassau grouper (Supplementary Fig. 6), highlighting a potential disconnect between the role of genetics to delineate population structure and connectivity. General awareness regarding the use of population genetics is not surprising, however, because it has not been traditionally applied in The Bahamas until fairly recently (Sherman et al. 2016, 2017). In contrast, research on spawning aggregations and evaluating the impacts of threats on Nassau grouper (more familiar types of research) were particularly important for enforcement stakeholders (Fig. 10). Approximately 93 % and 45 % of conservation and enforcement stakeholders, respectively, strongly agreed that science should be used to inform management for Nassau grouper (Fig. 10). However, in total, 20 % of enforcement stakeholders were in strong disagreement with this statement or selected “Don’t know” (Fig. 10). Aside from a lack of knowledge or awareness, this suggests that these stakeholders may have mistrust in the scientific process or could have misinterpreted the question. Previous studies have documented misconceptions or uncertainties over the role of science in informing marine policy and how confusion over survey questions could affect results and consequently, interpretations of the data (e.g., Mason et al. 2017).


a)
Count
16
14
12
10
8
6
4
2
0
Spawning aggregations
Pop. struct. & con.
Stock assessments
Migration patterns
Socioeconomic evaluation
Impacts of threats
Larval behaviour/ecology
Other


b)
Scientists communicate effectively
6 - Don't know
5 - Strongly disagree
4 - Disagree
3 - Neither agree or disagree
2 - Agree
1 - Strongly agree
0
20
40
60
80
100
Percentage (%)


c)
Trust in scientific data
6 - Don't know
5 - Strongly disagree
4 - Disagree
3 - Neither agree or disagree
2 - Agree
1 - Strongly agree
0
20
40
60
80
100
Percentage (%)
Conservation
Enforcement

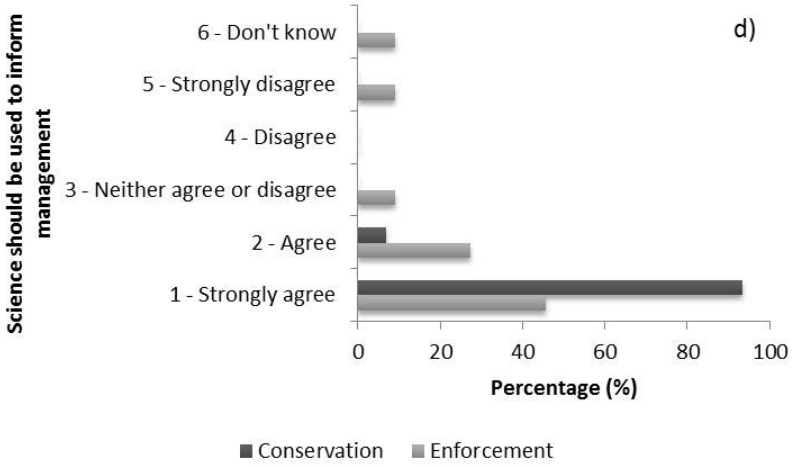

d)
Science should be used to inform management
6 - Don't know
5 - Strongly disagree
4 - Disagree
3 - Neither agree or disagree
2 - Agree
1 - Strongly agree
0
20
40
60
80
100
Percentage (%)
Conservation
Enforcement

Figure 10. Stakeholder perceptions regarding a) important research areas, b) ability of scientists to effectively communicate, c) trust in scientific data and d) the usefulness of science to inform management.

Similar approaches have been used for the fishing sector and the general public to assist with improving outreach for Nassau grouper management within the last five years (Supplementary Table 1). However, stakeholders had varied opinions regarding the success of these approaches, which were also reflected in the SWOT analysis (Table 1). Across both stakeholder groups only a few individuals (n=8) perceived them to be very effective. When asked what new approaches could be used to improve management for Nassau grouper, 81 % of stakeholders offered suggestions. Responses, however, were mainly recommendations to increase and/or sustain existing efforts (e.g., PSAs, television, social media), but a few new approaches also emerged e.g., aquaculture and development of site-specific management plans with fishers (Box 3). As an example, one stakeholder stated, “Sustained and targeted outreach to various stakeholders are required to stress the benefits of current and any proposed fishery regulations”. Hilborn et al. (2004) argued that both bottom-up and top-down approaches are required to achieve behavioural changes, which would be mutually beneficial for individuals and society. In the case of Nassau grouper, mostly top-down methods (i.e. through regulatory measures) have been applied to address challenges facing the fishery (Sherman et al. 2016), providing some explanation for the continued problem of illegal fishing.

Box 3. What new approaches can be used to improve management of Nassau grouper with a view to increase knowledge, change attitudes/perceptions and influence behaviour of fishers and the public?

"Provide training on how to obey the law. This may seem intuitive but it is not". — *Conservation stakeholder*

"Illegal capture or sale of Nassau grouper needs to have consequences for the offenders. The public & consumers (e.g., restaurants that buy fish) need to be informed of the rules more effectively". — *Conservation stakeholder*

"Start in one place, develop a relationship with fishers, involve them in research, form a site specific management plan for their fishing grounds, commit resources to making the plan happen". — *Conservation stakeholder*

"Encourage public discussion through avenues that utilize modern communication methods and using avenues through people are likely to view or talk about issues, such as Whatsapp". — *Conservation stakeholder*

"Implement a fish farm in a natural habitat". — *Enforcement stakeholder*

"Issue license to fishermen to sell fisheries products. In order to be issued a license you have to sit yearly workshops/seminars. So technically to get your license/permit you must do the workshop"! — *Enforcement stakeholder*

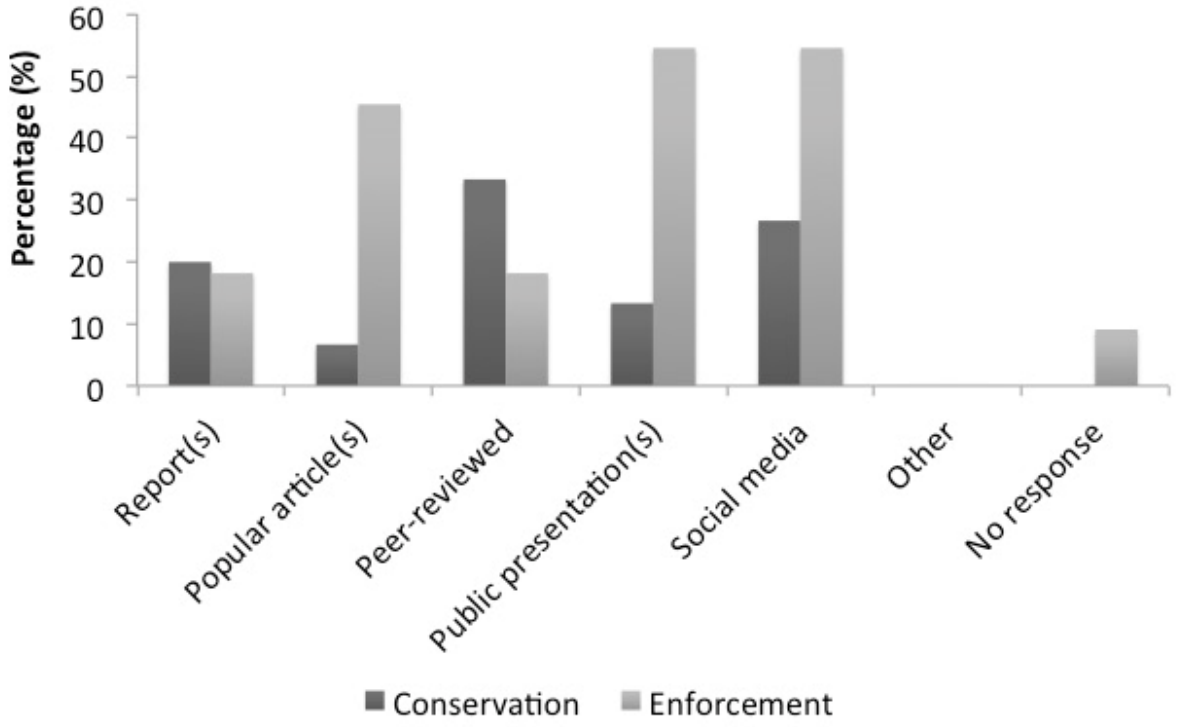


Figure 11. Preferred methods of communicating information stemming from Nassau grouper research to conservation and enforcement stakeholders.

Peer reviewed manuscripts and social media were selected as the top two methods for communicating information about Nassau grouper research for conservation stakeholders; whereas public presentations and social media were selected as the preferred options for enforcement stakeholders (Fig. 11). In The Bahamas, clarity, continuity, and consistency for enforcement and outreach efforts have been lacking due to funding/resource limitations, but other issues including political will, bureaucracy, and poor planning are also limiting factors (Table 1; Box 2). However, some of these issues can be compensated by 1) increased inter-agency cooperation to streamline management, enforcement and outreach efforts, 2) utilising free communication platforms (e.g., email and social media) to disseminate information, 3) diversifying sources of funding and 4) applying adaptive management approaches to monitor the efficacy of these initiatives (Wheat et al. 2013; Parsons et al. 2014; Wise 2014; Lewison et al. 2015; Sherman et al. In Review). Indeed, Wise (2014) demonstrated how failure of organisations to learn from past mistakes via an adaptive

management process delayed the establishment of MPAs in The Bahamas. More recently, social scientists have been advocating the use of the social-ecological system (SES) framework to promote sustainable resource management as it assimilates information from both fields (Leslie et al. 2015). However, such an approach was beyond the scope of the present research and merits exploration to determine its appropriateness for use in The Bahamas.

Conclusion

The present study aimed to explore stakeholder perspectives regarding Bahamian Nassau grouper. Through stakeholder assessments, we showed that the future sustainability of Nassau grouper in The Bahamas is of major concern for marine resource managers, educators, researchers and enforcement officers because of the cultural, economic and ecological importance of the species. While stakeholder views regarding the current status of Nassau grouper were variable and in some instances differ from available scientific data, perceived threats to the species were common along with a recognised need to improve existing management and education/outreach methods.

Evaluating and integrating stakeholder perspectives are important for implementing effective management strategies (Ciannelli et al. 2014; Singh et al. 2014; Mason et al. 2017), especially those tasked with advocacy and resource management. The Bahamian Nassau grouper fishery, while essential for many individuals, has proven difficult to effectively manage (Sherman et al. 2016). Although there has been increased recognition of the value of interdisciplinary research approaches to assist with marine resource management efforts in The Bahamas, social science has been largely neglected from this framework and/or the social component has been done after the fact (e.g., Stoffle and Minnis 2007, 2008; Wise 2014; Hayes et al. 2015). While preliminary in nature, due to low sample sizes and reduced geographic representation across The Bahamas, data from this research has shown that conservation and enforcement stakeholders have varied perceptions of the state of the Nassau grouper fishery and how it should be managed. Despite these differences, stakeholders demonstrated strong support for amendments to existing fishery regulations and the establishment of

new (science-based) regulations that would better protect Nassau grouper, along with increasing penalties for violating fishery regulations. Future research is required to expand stakeholder assessments, increasing diversification and representativeness utilising mixed approaches (e.g., fisher interviews, consumer surveys, etc.) to enhance understanding of socioeconomic drivers, thoroughly evaluate support for proposed changes to fishery regulations, and help to refine education/outreach efforts for better compliance with national management strategies. Such an approach should generate sufficiently large sample sizes, which would allow for more robust investigations and complex analyses of explanatory variables using models (e.g. Saldaña et al. 2016) or cluster analysis (e.g. Mason et al. 2017).

Overall, this research has illuminated several areas where additional work is required. Specifically, there is an urgent need to 1) increase capacity for enforcement through adequate funding, staff training and improve reporting systems, 2) correct inaccuracies that exist among some conservation and enforcement stakeholders regarding current fishery regulations and the status of the fishery, and 3) develop accurate yet diverse, targeted, and consistent communication messages for various stakeholder groups that will facilitate better compliance with national fishery regulations. This may be achieved through improved national and regional collaborations among government, marine resource managers, NGOs and scientists in conjunction with key stakeholders (e.g. fishers and the media). Combining both biological and socioeconomic data will be critical to help guide the development and implementation of a practical, scientifically based management plan for Nassau grouper that also considers the needs and perceptions of Bahamians. Failure to address these issues is likely to retard ongoing efforts to effectively manage the Bahamian Nassau grouper fishery.

Acknowledgements

We wish to thank L. Evans, R. Turner and J. Gardner Le Gars for useful input during the design of the questionnaires. Special thanks to A. Doerr and K. Maltby for advice and constructive critiques of earlier drafts of this work. Research was conducted under permit to K. Sherman from The Bahamas Department of Marine Resources and

with ethical approval from the Biosciences Department, University of Exeter. Results from this research will be shared will all participating stakeholders and the Department of Marine Resources to support national conservation management efforts for Nassau grouper. We have no conflicting interests to declare.

References

Cardinale BJ, Duffy JE, Gonzalez A, Hooper DU, Perrings C, Venail P, Narwani A, Mace GM, Tilman D, Wardle DA, Kinzig AP, Daily GC, Loreau M, Grace JB, Larigauderie A, Srivastava DS, Naeem S (2012) Biodiversity loss and its impact on humanity. Nature 486:59–67

Carpenter KE, Claro R, Cowan J, Sedberry G, & Zapp-Sluis M (2015) *Epinephelus striatus*. The IUCN Red List of Threatened Species 2015: e.T7862A70324790. Downloaded on 19 March 2018

Cheung WWL, Sarmiento JL, Dunne J, Frölicher TL, Lam VWY, Deng Palomares ML, Watson R, Pauly D (2012) Shrinking of fishes exacerbates impacts of global ocean changes on marine ecosystems. Nature Climate Change 3:254−258

Cheung, WWL, Sadovy Y, Braynen MT, Gittens LG (2013) Are the last remaining Nassau grouper *Epinephelus striatus* fisheries sustainable? Status quo in the Bahamas. Endangered Species Research 20:27−39

Ciannelli L, Hunsicker M, Beaudreau A, Bailey K, Crowder LB, Finley C, Webb C, Reynolds J, Sagmiller K, Anderies JM, Hawthorne D, Parrish J, Heppell S, Conway F, Chigbu P (2014) Transdisciplinary graduate education in marine resource science and management. ICES Journal of Marine Science 71:1047−1051

Cushion N, Sullivan-Sealey K (2008) Landings, effort and socio-economics of a small scale commercial fishery in The Bahamas. Proceedings of the Gulf and Caribbean Fisheries Institute 600:162−166

Dahlgren C, Sherman K, Lang J, Kramer PR, Marks K (2016) Bahamas coral reef report card, Vol. 1: 2011−2013. Nassau, Bahamas, http://www.agrra.org/wp-content/uploads/2016/05/Bahamas-2016-Coral-Reef-Report-Card.pdf

FAO (2016) Western Central Atlantic Fishery Commission. Sixteenth session of the Commission report No. 1162. Guadeloupe, 20–24 June 2016

Federal Register (2016) Endangered and Threatened Wildlife and Plants: Final listing determination on the proposal to list the Nassau grouper as threatened under the Endangered Species Act. Federal Register 81:42268–42285

Foley JA, DeFries R, Asner GP, Barford C, Bonan G, Carpenter SR, Chapin FS, Coe MT, Daily GC, Gibbs HK, Helkowski JH, Holloway T, Howard EA, Kucharik CJ, Monfreda C, Patz JA, Prentice IC, Ramankutty N, Snyder PK (2005) Global consequences of land use. Science 309:570–574

Forster J, Lake IR, Watkinson AR, Gill JA (2011) Marine biodiversity in the Caribbean UK overseas territories: Perceived threats and constraints to environmental management. Marine Policy 35:647–657

Giglio VJ, Bender MG, Zapelini C, Ferreira CEL (2017) The end of the line? Rapid depletion of a large-sized grouper through spearfishing in a subtropical marginal reef. Perspectives in Ecology and Conservation 15:115–118

Gringart E, Helmes E, Speelman C (2008) Harnessing cognitive dissonance to promote positive attitudes towards older workers in Australia. Journal of Applied Social Psychology 38:751–778

Hayes MC, Nils Peterson MN, Heinen-Kay JL, Langerhans RB (2015) Tourism-related drivers of support for protections of fisheries resources on Andros Island, The Bahamas

Helms MM, Nixon J (2010) Exploring SWOT analysis - where are we now?: A review of academic research from the last decade. Journal of Strategy and Management 3:215–251

Heppell SA, Semmens BX, Archer SK, Pattengill-Semmens CV, Bush PG, McCoy CM, Heppell SS, Johnson BC (2012) Documenting recovery of a spawning aggregation through size frequency analysis from underwater laser callipers measurements. Biological Conservation 155:119–127

Hicks CC, Crowder LB, Graham NAJ, Kittinger JN, Cornu EL (2016) Social drivers forewarn of marine regime shifts. Frontiers in Ecology and the Environment 14:252–260

Hilborn R (2007) Moving to sustainability by learning from successful fisheries. AMBIO: A Journal of the Human Environment 36:296–303

Hilborn R, Punt AE, Orensanz J (2004) Beyond band-aids in fisheries management: fixing world fisheries. Bulletin of Marine Science 493–507

Innes R, Polasky S, Tschirhart J (1998) Takings, compensation and endangered species protection on private land. Journal of Economic Perspectives 12:35–52

Jackson BC (2010) The future of oceans past. Philosophical Transactions of the Royal Society B: Biological Sciences 365: 3765–3778

Knowles JE, Green AL, Dahlgren C, Arnett F, Knowles L (2017) Expanding The Bahamas marine protected area network to protect 20% of the marine and coastal environment by 2020: a gap analysis. Nassau, Bahamas 66 pp.

Leslie HM, Basurto X, Nenadovic M, Sievanen L, Cavanaugh KC, Cota-Nieto JJ, Erisman BE, Finkbeiner E, Hinojosa-Arango G, Moreno-Báez M, Nagavarapu S, Reddy SM, Sánchez-Rodríguez A, Siegel K, Ulibarria-Valenzuela JJ, Weaver AH, Aburto-Oropeza O (2015) Operationalizing the social-ecological systems framework to assess sustainability. 112:5979–84

Levin N, Tulloch AIT, Gordon A, Mazor T, Bunnefeld N, Kark S (2017) Incorporating socioeconomic and political drivers of international collaboration into marine conservation planning. BioScience 63:547–563

Lewison R, Hobday AJ, Maxwell S, Hazen E, Hartog JR, Dunn DC, Briscoe D, Fossette S, O'Keefe CE, Barnes M, Abecassis M, Bograd S, Bethoney ND, Bailey H, Wiley D, Andrews S, Hazen L, Crowder LB (2015) BioScience 65:486–498

Mason JA, Rudd MA, Crowder LB (2017) Ocean research priorities: Similarities and differences among scientists, policymakers and fishermen in the United States. BioScience 67:418–428

Mora C, Myers RA, Coll M, Libralato S, Pitcher TJ, Sumaila RU, Zeller D, Watson R, Gaston KJ, Worm B (2009) Management effectiveness of the world's marine fisheries. PLoS Biology 7:e1000131

Oliveros, J.C. (2007-2015) Venny. An interactive tool for comparing lists with Venn's diagrams. http://bioinfogp.cnb.csic.es/tools/venny/index.html

Olsen DA, LaPlace JA (1979) A study of a Virgin Islands grouper fishery based on a breeding aggregation. Proceedings of the Gulf and Caribbean Fisheries Institute. 31:130–144

Parfitt, J (2005) Questionnaire design and sampling, in: Methods in Human Geography, edited by: Flowerdew, R. and Martin, D., Pearson Education Limited, England, 78–109

Pauly D, Zeller D (2016) Catch reconstructions reveal that global marine fisheries catches are higher than reported and declining. Nature Communications 7:10244, doi:10.1038/ncomms10244

Pauly D, Zeller D (2017) Comments on FAOs State of World Fisheries and Aquaculture (SOFIA 2016). Marine Policy 77:176–181

Parson ECM, Shiffman DS, Darling ES, Spillman N and Wright AJ, (2014), How Twitter Literacy Can Benefit Conservation Scientists. Conservation Biology, 28: 299–301

Pomeroy R, Parks J, Mrakovich KL, LaMonica C (2016) Drivers and impacts of fisheries scarcity, competition, and conflict on maritime security. Marine Policy 67:94–104

Purcell SW, Pomeroy RS (2015) Driving small-scale fisheries in developing countries. Frontiers in Marine Science https://doi.org/10.3389/fmars.2015.00044

Ray GC (2000) Investigations of Nassau grouper breeding aggregations at High Cay, Andros: implications for a conservation strategy. Final Report, Department of Fisheries, Nassau

Robinson J, Graham NAJ, Cinner JE, Almany GR, Waldie P (2015) Fish and fisher behaviour influence the vulnerability of groupers (Epinephelidae) to fishing at a multispecies spawning aggregation site. Coral Reefs 34: 371–382

Robinson J, Samoilys MA (2013) (Co-editors) (2013) Reef fish spawning aggregations in the Western Indian Ocean: research for management. WIOMSA/SIDA/SFA/CORDIO. WIOMSA Book Series 13.

Sadovy Y, Eklund AM (1999) Synopsis of biological data on the Nassau grouper, *Epinephelus striatus* (Bloch, 1792) and the Jewfish, *E. itajara* (Lichtenstein, 1822). NOAA Tech Rep NMFS 146

Sadovy de Mitcheson Y, Colin PL (eds.) (2012) Reef Fish Spawning Aggregations: Biology, Research and Management. Vol. 35. Fish and Fisheries Series. Springer, New York 621 pp.

Sadovy de Mitcheson YS, Erisman B (2012) Fishery and biological implications of fishing spawning aggregations, and the social and economic importance of aggregating fishes. In: Sadovy de Mitcheson Y, Colin PL (eds) Reef fish spawning aggregations: biology, research and management. Springer, New York, NY, p 225−284

Sadovy de Mitcheson Y, Craig MT, Bertoncini AA, Carpenter KE, Cheung WLL, Choat JH, Cornish AS, Fennessy ST, Ferreira BP, Heemstra PC, Liu M, Myers RF, Pollard DA, Rhodes KL, Rocha LA, Russell BC, Samoilys MA, Sanciangco J (2013) Fishing groupers towards extinction: a global assessment of threats and extinction risks in a billion dollar fishery. Fish and Fisheries 14:119–136

Saenz-Arroyo A, Roberts CM, Torre J, Carino-Olvera M, Enriquez-Andrade RR. 2005. Rapidly shifting environmental baselines among fishers of the Gulf of California. Proceedings of the Royal Society B 272:1957–1962

Sala E, Ballesteros E, Starr RM (2001) Rapid decline of Nassau grouper spawning aggregations in Belize: fishery management and conservation needs. Fisheries 26: 23–30

Saldaña A, Salas S, Arce-Ibarra AM, Torres-Irineo E (2016) Fishing operations and adaptive strategies of small-scale fishers: insights for fisheries management in data-poor situations. Fisheries Management and Ecology doi:10.1111/fme.12199

Selkoe KA, Blenckner T, Caldwell MR, Crowder LB, Erickson AL, Essington TE, Estes JA, Fujita RM, Halpern BS, Hunsicker ME, Kappel CV, Kelly RP, Kittinger JN, Levin PS, Lynham JM, Mach ME, Martone RG, Mease LA, Salomon AK, Samhouri JF,

Scarborough C, Stier AC, White C, Zedler J (2015) Principles for managing marine ecosystems prone to tipping points. Ecosystem Health and Sustainability 1: 1–18

Shahidul Islam M, Tanaka M (2004) Impacts of pollution on coastal and marine ecosystems including coastal and marine fisheries and approach for management: a review and synthesis. Marine Pollution Bulletin 48:624–649

Sherman KD, Dahlgren CP, Knowles LC (2018) Nassau Grouper (*Epinephelus striatus*) conservation management plan for The Commonwealth of The Bahamas. Prepared for the Department of Marine Resources, Nassau, Bahamas

Sherman KD, Dahlgren CP, Stevens JR, Tyler CR (2016) Integrating population biology into conservation management for endangered Nassau grouper *Epinephelus striatus*. Marine Ecology Progress Series 554:263–280

Sherman KD, King RA, Dahlgren CP, Simpson SD, Steven JR, Tyler CR (2017) Historic and contemporary anthropogenic activities influence genetic population dynamics of Nassau grouper (*Epinephelus striatus*) within The Bahamas. Frontiers in Marine Science 4:393. doi:10.3389/fmars.2017.00393

Sherman KD, Shultz AD, Dahlgren CP, Thomas C, Brooks E, Brooks A, Brumbaugh DR, Gittens L, Murchie K (In Press) Contemporary and emerging fisheries in The Bahamas – conservation and management challenges achievements and future directions. Fisheries Management and Ecology doi: 10.1111/fme.12299

Singer E, Couper MP (2008) Do incentives exert undue influence on survey participation? Experimental evidence. Journal of Empirical Research and Human Research Ethics 3:49–56

Singh GG, Tam J, Sisk TD, Klain SC, Mach ME, Martone RG, MA Chan K (2014) A more social science: barriers and incentives for scientists engaging in policy. Frontiers in Ecology and the Environment 2014: doi:10.1890/130011

Smith CL (1972) A spawning aggregation of Nassau grouper, *Epinephelus striatus* (Bloch). Transactions of the American Fisheries Society 101:257–261

Stoffle R, Minnis J (2007) Marine protected areas and the coral reefs of traditional settlements in the Exumas, Bahamas. Coral Reefs 26:1023–1032

Stoffle R, Minnis J (2008) Resilience at risk: epistemological and social construction barriers to risk communication. Journal of Risk Research 11:55–68

Stump K, Dahlgren CP, Sherman KD, Knapp CR (2017) Nassau grouper migrations during full moon suggest collapsed historic fish spawning aggregation and evidence of undocumented aggregation. Bulletin of Marine Science 93:375–389

The Bahamas Maritime Authority (2017) Illegal, unreported and unregulated (IUU) fishing and authorisation to conduct transshipment operations. Guidance and Instruction for Bahamas ship owners, managers, masters and approved nautical inspectors. Bulletin No. 155. Revision No. 4. Nassau, Bahamas

Turner RA, Fitzsimmons C, Forster J, Mahon R, Peterson A, Stead SM (2016) Measuring good governance for complex ecosystems: perceptions of coral reef-dependent communities in the Caribbean. Global Environmental Change 29-105–117

Turner RA, Polunin NVC, Stead SM (2014) Social networks and fishers' behaviour: exploring the links between information flow and fishing success in the Northumberland lobster fishery. Ecology and Society 19:38, doi:10.5751/ES-06456-190238

Westhues A, Lafrance J, Schmidt G (2010) A SWOT analysis of social work education in Canada. Social Work Education: The International Journal 20:35–56

Whaylen L, Pattengill-Semmens CV, Semmens BX, Bush PG, Boardmand MR (2004) Observations of a Nassau grouper, *Epinephelus striatus*, spawning aggregation site in

Little Cayman, Cayman Islands, including multispecies spawning information. Environmental Biology of Fishes 70:305−313

Whaylen L, Bush PG, Johnson BC, Luke KE, McCoy C, Heppell S, Semmens B, Boardman M (2007) Aggregation dynamics and lessons learned from five years of monitoring at a Nassau grouper (*Epinephelus striatus*) spawning aggregation in Little Cayman, Cayman. Proceedings of the Gulf and Caribbean Fisheries Institute 59:1−14

Wheat RE, Wang Y, Byrnes JE, Ranganathan J (2013) Raising money for scientific research through crowdfunding. Trens in Ecology and Evolution 28:71−72

Wilson RS, Hardisty DJ, Epanchin-Niell RS, Runge MC, Cottingham KL, Urban DL, Maguire LA, Hastings A, Mumby PJ, Peters DPC (2016) A typology of time-scale mismatches and behavioral interventions to diagnose and solve conservation problems. Conservation Biology 30:42−49

Wise SP (2014) Learning through experience: Non-implementation and the challenges of protected area conservation in The Bahamas. Marine Policy 46:111−118

Yasué M, Kaufman L, Vincent ACJ (2010) Assessing ecological changes in and around marine reserves using community perceptions and biological surveys. Aquatic Conservation: Marine and Freshwater Ecosystems 20:407–418

Supplementary Materials

Stakeholders Perspectives on the Status and Conservation Management of Nassau grouper in The Bahamas

Krista D. Sherman[1,2,3*] and Charles R. Tyler*[1]

Correspondence:

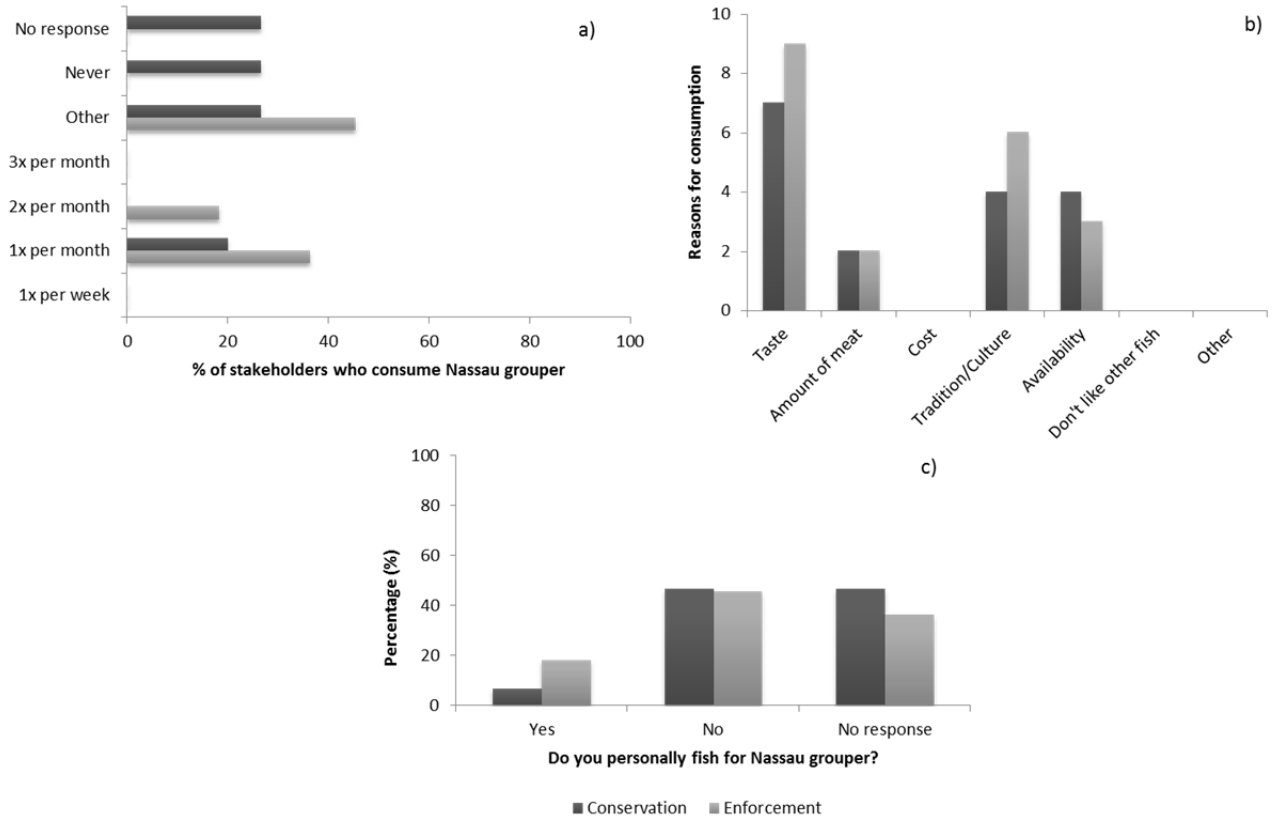
Krista Sherman
kds204@exeter.ac.uk or krista.daniellesherman@gmail.com

Professor Charles Tyler
C.R.Tyler@exeter.ac.uk

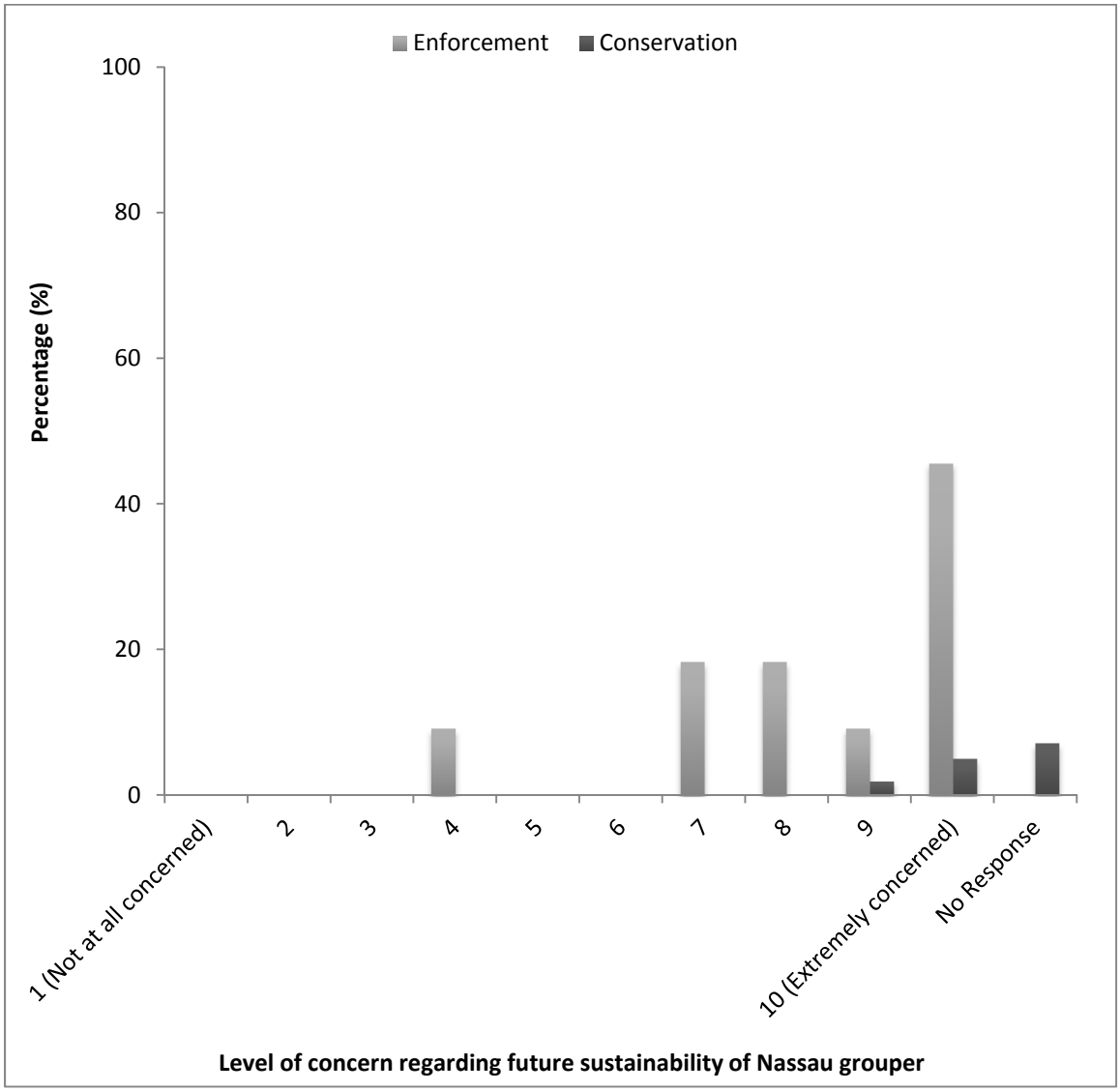
List of Figures

List of Tables

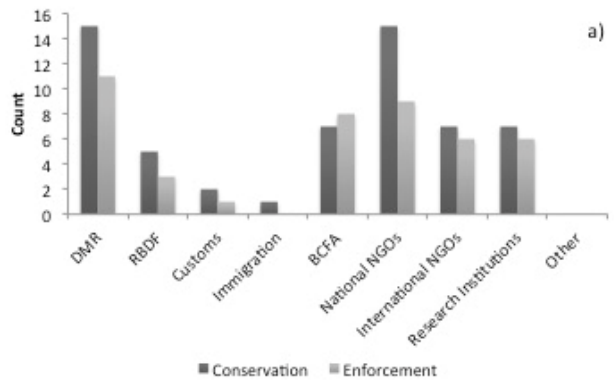
Supplementary Fig. 1

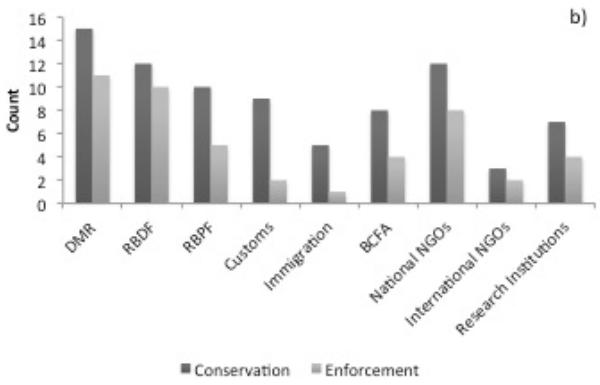


Supplementary Fig. 2

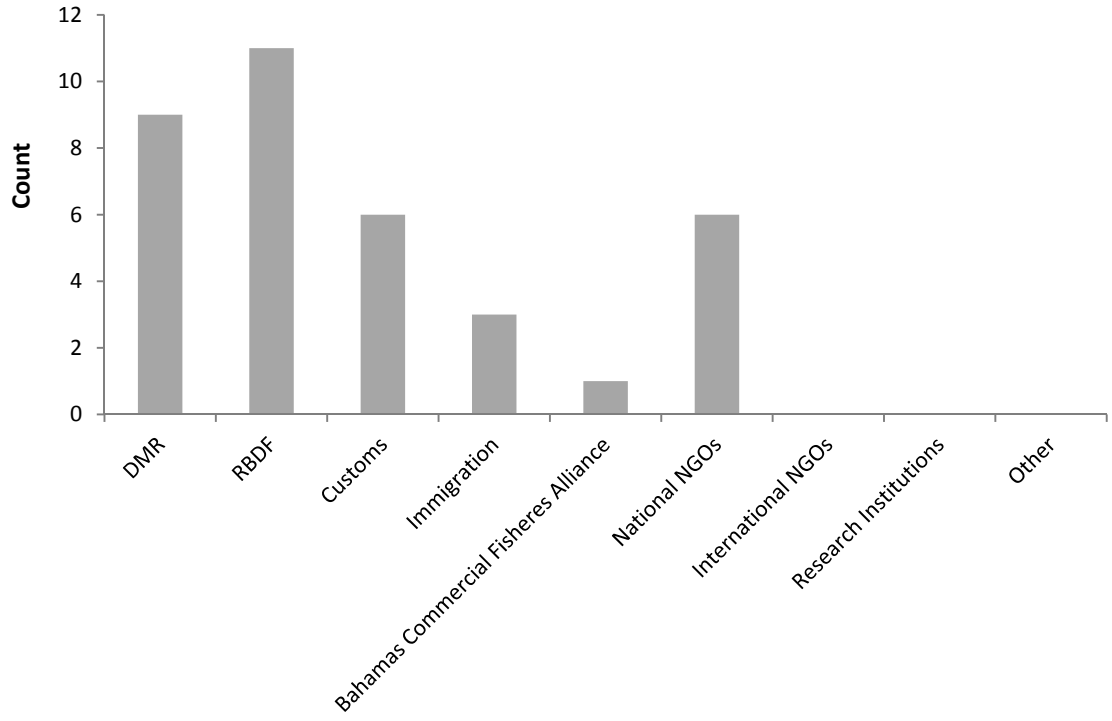


Supplementary Fig. 3

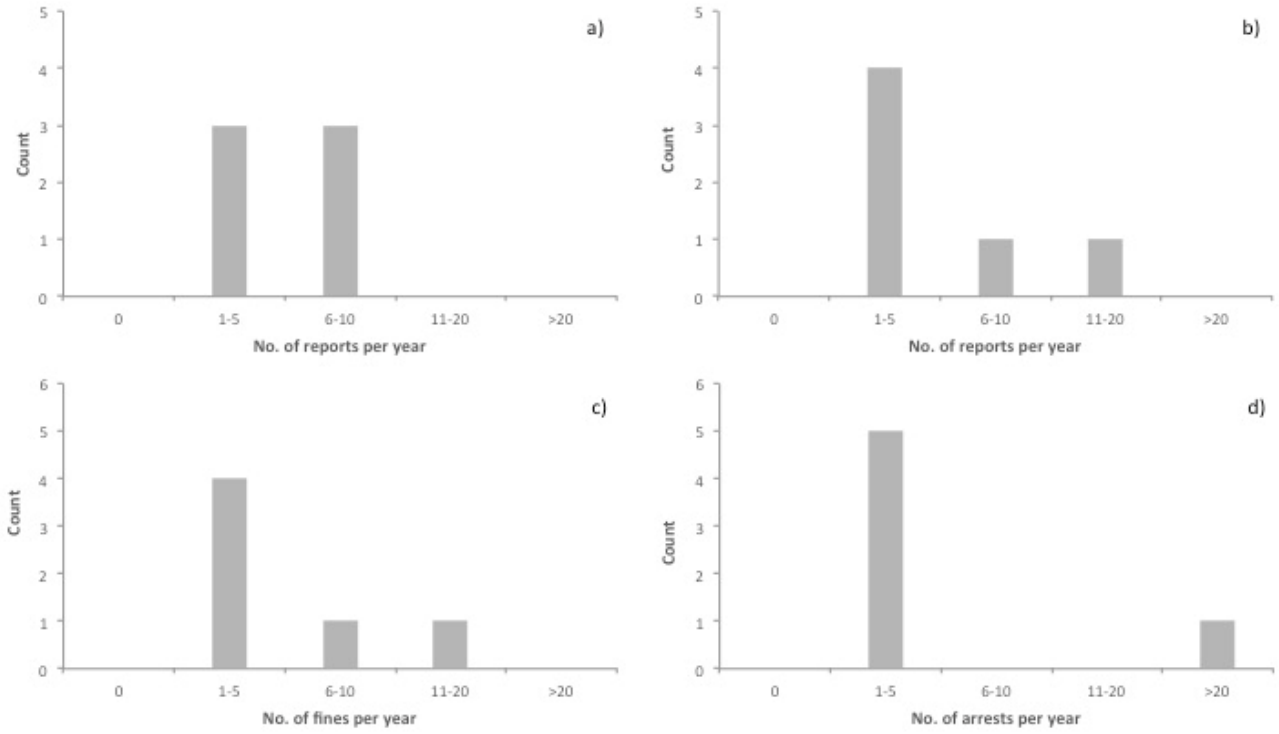




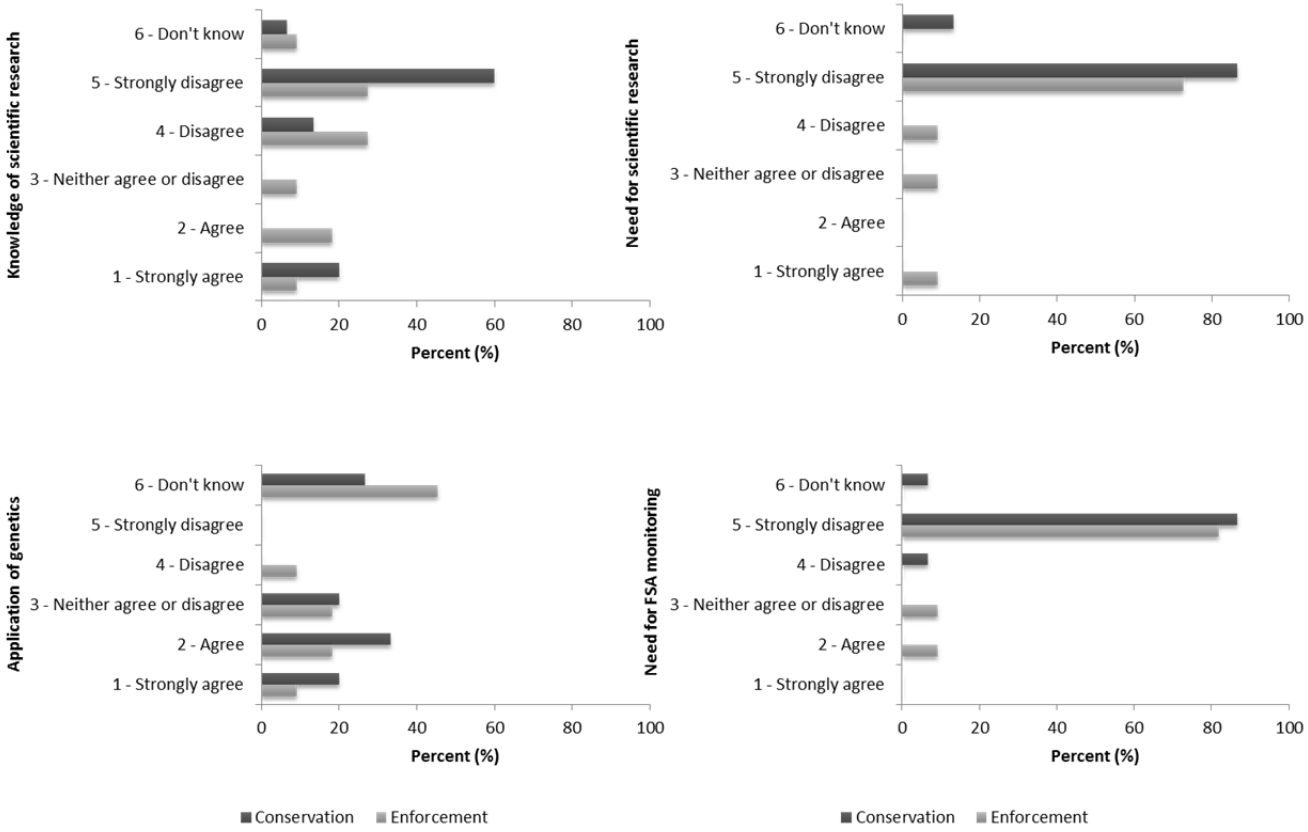
Supplementary Fig. 4



Supplementary Fig. 5



Supplementary Fig. 6



Supplementary Table 1.

Fisheries Sector	General Public
Direct communication	Direct communication
Documentary/videos	Diver specialty programmes
Fishing rules app	Documentary/videos
Meetings	Educational materials/programmes for children
More military presence	Meetings
MPAs	NGOs working with DMR
PSAs	Press releases
Signs, posters, brochures	PSAs
Social media	Public presentations
TV, Radio and newspaper ads	Signs, posters, brochures
Workshops	Social media
	Teacher training workshops
	TV, Radio and newspaper ads

Supplementary Material 1 – Facilitator Instructions

Nassau Grouper Fishery Management Workshop

Instructions for facilitators:

Each facilitator is responsible for managing time and recording outcomes from your assigned stakeholder group's discussion.

1) Complete SWOT analysis with your group (10 min).
2) To begin ask your break-out group question number 1.
3) Give each person 5 minutes to write down their responses on the provided post-it notes for questions 2-6.
4) Share /discuss responses and collate using the labelled flip-charts provided.
5) Get a volunteer from your group to share prioritized responses for the larger/general group discussion (time allotted for this = 20 min).

We will spend 30 min addressing workshop objectives 3-4.

1) Ask your groups to comment on the proposed activities, timeline and components for the DRAFT management plan and indicate areas where they are willing & able to committing support (in-kind or financial).
2) Collate this information using an Excel spreadsheet. Data from each will be merged into one document and shared with stakeholders after the workshop.

If you complete your SWOT analysis before we return to the general group discussion, use the other questions provided to stimulate discussion and gather additional input from your assigned stakeholder group.

Provided Materials – Flip chart, markers, post-it notes, pens

Discussion Questions/Prompts

Group 1 - Fishermen, BCFA, Wholesalers, Fish Processing Plants (BMEA, Tropic Seafood, Paradise Fisheries)

1. How would you characterize/describe (e.g. status, primary gears used, no. involved) the Nassau grouper fishery in your island? in The Bahamas?
2. In your opinion what are the strengths/benefits of the Nassau grouper fishery?
3. How would your rank/prioritize these strengths/benefits?
4. In your opinion what are the threats/issues facing the Nassau grouper fishery?
5. How would you rank/prioritize these threats/issues?
6. Do you have any recommendations on how these threats/issues can be addressed?
7. What are the pros and cons of the closed season?

8. How does the closed season affect fishermen in your island/Bahamas?
9. Were you aware of all fishing regulations pertaining to Nassau grouper? If not, which of the regulations presented today were new to you?
10. How many of the fishermen in your island are knowledgeable about Nassau grouper fishery regulations?
11. How many of fishermen in your community support and comply with these regulations?
12. What other options available to the fishermen in your community to earn money during the closed season?
13. How can policy-makers, law enforcement, scientists, marine resource managers help the fishing community?
14. Which organizations should be managing the fishery?
15. Describe the importance of the Nassau grouper fishery to you/your company (i.e. revenue generated (from local consumers, exports, etc., no. of staff employed)
16. Do you have any new recommendations that would improve fisheries management for Nassau grouper?
17. What is the best way for information on Nassau grouper (research, education, policy) to be communicated to fishermen, fish processing plants, consumers, etc.?
18. What additional/new information would you need to support changes to existing regulations or the creation of new regulations?

Group 2 - Enforcement Officials (Fisheries Officers, RBDF, RBPF, BNT Wardens, Customs, Immigration)

1. How would you characterize/describe (e.g. status, primary gears used, no. involved) the Nassau grouper fishery in your island? in The Bahamas?
2. In your opinion what are the strengths/benefits of the Nassau grouper fishery?
3. How would your rank/prioritize these strengths/benefits?
4. In your opinion what are the threats/issues facing the Nassau grouper fishery?
5. How would you rank/prioritize these threats/issues?
6. Do you have any recommendations on how these threats/issues can be addressed?
7. Were you aware of all fishing regulations pertaining to Nassau grouper? If not, which of the regulations presented today were new to you?
8. Do the RBDF, RBPF, Customs & Immigration have a training program for its officers that covers fisheries regulations, locations and rules of no-take marine reserves/MPAs?
9. If so how often is the material revised and delivered? If not, would developing this type of program be useful for your organizations?
10. What other resources/materials would be useful for your organizations to support staff training?
11. Are the current available resources sufficient to enforce fishery & MPA regulations?
12. What are the difficulties your respective organizations face with enforcing fisheries regulations?
13. Have there been any attempts to resolve these issues and if so what was the outcome?

14. What additional/new information would you require to better enforce fisheries regulations and how should this information be provided?
15. How can policy-makers, scientists, marine resource managers, etc. help to better support you in your role as law enforcement officers?
16. How often are violators of fisheries regulations prosecuted (i.e. percentage detected, fined, charged)?
17. In your opinion, are the penalties for violators sufficient?
18. From an enforcement perspective, do you have any new recommendations that would improve fisheries management for Nassau grouper?
19. What information would you need to support changes to existing regulations or the creation of new regulations?
20. Which organizations should be managing the fishery?

Group 3 - NGOs, Educators, Consultants (BNT, BREEF, CEI, TNC)

1. How would you characterize/describe (e.g. status, primary gears used, no. involved) the Nassau grouper fishery in your island? in The Bahamas?
2. In your opinion what are the strengths/benefits of the Nassau grouper fishery?
3. How would your rank/prioritize these strengths/benefits?
4. In your opinion what are the threats/issues facing the Nassau grouper fishery?
5. How would you rank/prioritize these threats/issues?
6. Do you have any recommendations on how these threats/issues can be addressed?
7. Were you aware of all fishing regulations pertaining to Nassau grouper? If not, which of the regulations presented today were new to you?
8. What are the current approaches/practices being used to help improve outreach and advocacy for Nassau grouper conservation? How effective have these approaches/practices been?
9. What new/innovative approaches can be used to improve advocacy for Nassau grouper conservation with a view to increasing knowledge, changing attitudes/perceptions and influencing behaviour?
10. What is the timeline for implementation?
11. How will you assess the effectiveness of these approaches?
12. How can your organizations improve upon the support provided to fishers, policy-makers, law enforcement, scientists, marine resource managers and each other?
13. Do you have any new recommendations that would improve fisheries management for Nassau grouper?
14. What is the best way for information on Nassau grouper research to be communicated to your organizations?
15. What additional/new information would you need to support changes to existing regulations or the creation of new regulations?

Group – 4 Potential Funders/Supporters (International SeaKeepers Society, Moore Bahamas Foundation)

1. How would you characterize/describe (e.g. status, primary gears used, no. involved) the Nassau grouper fishery in The Bahamas?
2. In your opinion what are the strengths/benefits of the Nassau grouper fishery?
3. How would your rank/prioritize these strengths/benefits?
4. In your opinion what are the threats/issues facing the Nassau grouper fishery?
5. How would you rank/prioritize these threats/issues?
6. Do you have any recommendations on how these threats/issues can be addressed?
7. Were you aware of all fishing regulations pertaining to Nassau grouper? If not, which of the regulations presented today were new to you?
8. What aspects of Nassau grouper conservation are you most interested in supporting and why?
9. What role can/will your organizations play to help provide support for on-going research, monitoring, outreach and advocacy?
10. Do you have any new recommendations that would improve fisheries management for Nassau grouper?

Supplementary Material 2 – Conservation Stakeholder Questionnaire

Project Overview:

Nassau grouper are in decline globally. This iconic economically and ecologically important species is listed as endangered on the IUCN Red List and has also recently been listed as "threatened" under the United States Endangered Species Act (ESA). Management and conservation have relied heavily on biological (i.e. monitoring data) and commercial fisheries landings data to assess numbers or stock size of Nassau grouper. Population genetics offers another biological approach to more accurately assess the status of Nassau grouper in The Bahamas. I will be using both underwater visual surveys of spawning aggregations and genetic techniques to analyze the health of Nassau grouper in The Bahamas, provide estimates of the number of breeders and identify the number of distinct populations present.

This information will provide the basis for a comprehensive management strategy for Nassau grouper along with considerations of other key aspects of the species' biology and ecology. However, socioeconomic factors as well as biological factors need to be assessed to effectively manage the Nassau grouper fishery. Focus group meetings and questionnaires will be used to assess stakeholder knowledge and justify the social and economic importance of Nassau grouper to The Bahamas. Combined biological and socioeconomic data will help to guide the development of a practical, scientifically based management plan for Nassau grouper that considers the needs and perceptions of Bahamians.

Please complete and return this questionnaire to Krista Sherman of the University of Exeter. The questionnaire should take 25-30 minutes to complete. When you do this you will be supporting efforts to evaluate the status and provide recommendations to promote a sustainable Nassau grouper fishery for The Bahamas. This questionnaire helps fulfill a socioeconomic gap in our knowledge of this hugely important resource.

The researcher carrying out this project is Krista Sherman who presented her work at the Bahamas Natural History Conference on March 16th, 2016. The project aims to take biological and socioeconomic approaches to provide appropriate management measures for Nassau grouper.

Please take the time to fill in this questionnaire and return it by **February 28th, 2017**.

Via

1. Email to kds204@exeter.ac.uk with 'Nassau Grouper Questionnaire' in the title.
2. Post to Krista Sherman, University of Exeter, Biosciences, Lab 201, Stocker Road, EX4 4QD, UK.

Completion of this questionnaire is voluntary and all returned questionnaires will be coded for your anonymity.

Consent Form – Confidential Data

I understand that my participation in this project is voluntary and that I can withdraw at any time without providing a reason.

I understand that all information provided by me will remain confidential and that my name will not be directly associated with any data I provide.

I understand that I can ask for the information I provide to be deleted/destroyed at any time and that I can have access to my information.

I understand that I can omit (not answer) questions that I do not wish to answer.

I also understand that at the end of the project I will be provided with a copy of the outputs.

I, _____**(PRINT NAME)** consent to participate in this research led by Krista Sherman at the University of Exeter.

Signed: _____ Date: _____

The Bahamian Nassau Grouper Fishery

Section 1. Knowledge & Perceptions of Environmental Change

Q1. Using the scale provided, please **circle** the number that best reflects your understanding of the current state of Nassau grouper numbers (abundance) on **reefs**.

1	2	3	4	5
Significant decline	Decline	no change	Increase	Significant increase

Q2. Using the scale provided, please **circle** the number that best reflects your understanding of the current state of Nassau grouper numbers (abundance) in **nursery habitats (e.g. mangroves and seagrasses)**.

1	2	3	4	5
Significant decline	Decline	no change	Increase	Significant increase

Q3. Using the scale provided, please **circle** the number that indicates your knowledge about changes in the numbers of Nassau grouper caught (landed) in The Bahamas over the last 10 years.

1	2	3	4	5
Significant decline	Decline	no change	Increase	Significant increase

Q4. For your responses to questions 1-3, how do you know this? *Please select all that apply.*

	Q1	Q2	Q3
Brochure/flyer	☐	☐	☐
Poster	☐	☐	☐
Environmental documentary	☐	☐	☐
Scientific journal	☐	☐	☐
News	☐	☐	☐
Social Media	☐	☐	☐
Personal observation	☐	☐	☐
Other _____	☐ _____	☐ _____	☐ _____ (*please specify*)

Q5. Please list the top 3-5 reasons why you believe there has been a decline/increase in Nassau grouper in The Bahamas.

1. _____

2. _____

3. _____

4. _____

5. _____

Q6. Based on your understanding, please list the top 5 threats to the Nassau grouper in The Bahamas in order of importance (i.e. from 1 most important to 5 least important threat).

1. _____

2. _____

3. _____

4. _____

5. _____

Q7. On a scale from 1 (not at all concerned) to 10 (extremely concerned), please indicate your level of concern about the future sustainability of Nassau grouper in The Bahamas. *Please circle **one** number.*

1	2	3	4	5	6	7	8	9	10
Not at all concerned									Extremely concerned

Q8. Please list the top 3-5 reasons why you believe it is or is not important to conserve (sustainably manage) Nassau grouper.

1. _____

2. _____

3. _____

4. _____

5. _____

Section 2. Knowledge and Perceptions of Fishery Management in The Bahamas

Q9. Which of the following is the closed season for Nassau grouper? *Check **one** response.*

a. Dec 1 – Jan 31 ◯
b. Nov 1 – Dec 30 ◯
c. Dec 1 – Feb 28 ◯

Q10. What is the minimum size limit for fishing Nassau grouper? *Circle **one** response.*

a. 3 lb. ◯ b. 4 lb. ◯ c. 5 lb. ◯

Q11. Please rank the effectiveness of the following current methods for managing Nassau grouper (1=Very ineffective; 2=Ineffective; 3=Neither effective nor ineffective; 4=Effective; 5=Very effective; 6=Don't know). *Check the **one** answer for each method.*

	1	2	3	4	5	6
I. Minimum size limit	☐	☐	☐	☐	☐	☐
II. Closed season	☐	☐	☐	☐	☐	☐
III. Marine Protected Areas (MPAs)	☐	☐	☐	☐	☐	☐
IV. No-take MPAs or reserves	☐	☐	☐	☐	☐	☐

Q12. For each of your responses to **Q11**, please explain why you consider these methods as effective or not effective.

I.

II.

III.

IV.

Q13. Use the scale below to reflect how the current methods for managing Nassau grouper affect Bahamian fishers. 1=Very negative; 2=Negative; 3=Neither negative nor positive; 4=Positive; 5=Very positive; 6 = Don't know. *Check the **one** answer for each method.*

	1	2	3	4	5	6
I. Minimum size limit	☐	☐	☐	☐	☐	☐
II. Closed season	☐	☐	☐	☐	☐	☐
III. Marine Protected Areas (MPAs)	☐	☐	☐	☐	☐	☐
IV. No-take MPAs or reserves	☐	☐	☐	☐	☐	☐

Q14. Explain your responses in **Q13**. How do Nassau grouper fisheries regulations affect Bahamian fishermen?

I.

II.

III.

IV.

Q15. Please list the 3 main barriers to effective management of the Nassau grouper fishery in The Bahamas. Rank your top 3 responses (from 1 most important to 3 least important barriers).

1.

2.

3.

Q16. For each main barrier you listed in **Q15**, provide **one** way in which that barrier can be addressed (corrected/changed) to more effectively manage the Nassau grouper fishery in The Bahamas.

1.

2.

3.

Q17. In your opinion, what is the best method/strategy to improve sustainability of the Bahamian Nassau grouper fishery? *Please explain your answer.*

Q18 a. Would you support changes to existing regulations and **b.** the creation of new regulations to promote recovery of the Nassau grouper fishery?

a. Yes ☐ No ☐ **b.** Yes ☐ No ☐

Q19. Choose the amendments or changes to existing regulations that you would be willing to support. *Select **all** that apply.*

Increased minimum size limit ☐
Extended closed season ☐
New Marine protected areas (MPAs) ☐
New no-take MPAs or reserves ☐

Q20. Which of the following new regulations would you be willing to support? *Select **all** that apply.*

Establish quotas/bag limits ☐
Protection of fish spawning aggregation sites (FSAs) ☐
Ban use of hookahs (compressor diving) ☐
Ban use of traps/fish pots at FSAs during the closed season ☐
Establish maximum size limit ☐
New No-take MPAs or reserves ☐
Temporary ban (e.g. 10 yrs.) on fishing Nassau grouper ☐
Catch shares for fishers ☐
Other _____ (Please specify)

Q21. Please rate the following potential drivers/causes for illegal fishing (i.e. catching undersized fish or fishing during the closed season) from 1=Most important to 5=Least important.

	1	2	3	4	5
Poverty	☐	☐	☐	☐	☐
Access rights to fishing grounds	☐	☐	☐	☐	☐
Consumer demand	☐	☐	☐	☐	☐
Lack of knowledge of fishery regulations	☐	☐	☐	☐	☐
Lack of economic alternatives	☐	☐	☐	☐	☐
Population growth	☐	☐	☐	☐	☐
Foreign recreational fishers	☐	☐	☐	☐	☐
Foreign commercial fishers	☐	☐	☐	☐	☐
Corruption/bribery	☐	☐	☐	☐	☐

Q22. Based on your knowledge, please **select all the organizations** that are involved in making decisions about Nassau grouper management?

Department of Marine Resources ☐
Royal Bahamas Defence Force ☐
Customs ☐
Immigration ☐
Bahamas Commercial Fishers Alliance ☐
National Non-governmental organizations (NGOs) (e. g. BNT) ☐
International NGOs (e.g. TNC) ☐
Research Institutions (e.g. CEI) ☐
Other _____ ☐

Q23. In your opinion, which organizations *should* be **managing** the fishery? *Select **all** that apply.*

Department of Marine Resources ☐
Royal Bahamas Defence Force ☐
Royal Bahamas Police Force ☐
Customs ☐
Immigration ☐
Bahamas Commercial Fishers Alliance ☐
National Non-governmental organizations (NGOs – e.g. BNT) ☐
International NGOs ☐
Research Institutions ☐

Q24. *Please rank the following **organizations** in terms of whether or not they should have a say in management decisions?? 1-6 (1 should have the most say) or 0 (no say).*

Department of Marine Resources	Select
Royal Bahamas Defence Force	Select
Royal Bahamas Police Force	Select
Bahamas National Trust	Select
Customs	Select
Immigration	Select
Bahamas Commercial Fishers Alliance	Select

Q25. Do you have any other recommendations that would improve fisheries management for Nassau grouper? **(Max 100 words)**

Section 3. Science, Education & Outreach

Q26. Please list 3-4 current approaches/practices (i.e. within the last 5 yrs.) being used to help improve outreach to the **a.** fishing sector and **b.** general public for Nassau grouper management?

a. Fishing Sector

1. _____

2. _____

3. _____

4. _____

b. General Public

1. _____

2. _____

3. _____

4. _____

Q27. Please prioritize and rate the effectiveness (1=Very ineffective; 2= Ineffective; 3= Neither effective nor ineffective; 4= Effective; 5= Very effective) of approaches/practices you listed in **Q26** for a. the fishing sector and b. general public.

a. Fishing Sector

Approaches	1	2	3	4	5
1.	☐	☐	☐	☐	☐
2.	☐	☐	☐	☐	☐
3.	☐	☐	☐	☐	☐
4.	☐	☐	☐	☐	☐

b. General Public

Approaches	1	2	3	4	5
1.	☐	☐	☐	☐	☐
2.	☐	☐	☐	☐	☐
3.	☐	☐	☐	☐	☐
4.	☐	☐	☐	☐	☐

Q28. What new approaches can be used to improve management of Nassau grouper with a view to increase knowledge, change attitudes/perceptions and influence behaviour of fishers and the public? **(Max 100 words)**

Q29. How will you know if these approaches worked? **(Max 100 words)**

Q30. How quickly should these changes be implemented? *Select **one** response.*

Immediately ☐
Within 6 mo ☐
Within 1 yr ☐
Within 2-5 yrs ☐
Within6-10 yrs ☐

Q31. How can your organization improve upon the support provided to fishers, policy-makers, law enforcement, scientists, marine resource managers and other organizations? **(Max 100 words)**

Q32. What is the best way for information on Nassau grouper research to be communicated to your organizations?

Report(s) ☐, Popular article(s) ☐, Peer-reviewed manuscript(s) ☐, Public presentation(s) ☐
Social media ☐, Other _____ (please specify)

Q33. What additional/new information would you need to support changes to existing regulations or the creation of new regulations?

Q34. Is there an area of Nassau grouper research that is particularly important? *Select **all** applicable answers.*

Spawning aggregations ☐
Population structure and connectivity ☐
Stock assessments ☐
Migration patterns ☐
Socioeconomic evaluation of the fishery ☐
Impacts of threats ☐
Larval behaviour/ecology ☐
Other: _____ (Please specify)

Q35. Please respond to the following statements (1 =strongly agree; 5=strongly disagree; 6=don't know). *Select **one** response for each statement.*

	1	2	3	4	5	6
1. I am unaware of scientific research on Nassau grouper.	☐	☐	☐	☐	☐	☐
2. Scientific research on Nassau grouper is unnecessary.	☐	☐	☐	☐	☐	☐
3. Genetics is a useful tool to assess numbers of Nassau grouper.	☐	☐	☐	☐	☐	☐
4. Monitoring fish spawning aggregations is unnecessary.	☐	☐	☐	☐	☐	☐
5. Scientists communicate their results effectively.	☐	☐	☐	☐	☐	☐
6. I do not trust scientific information/data.	☐	☐	☐	☐	☐	☐
7. Science should be used to inform management.	☐	☐	☐	☐	☐	☐

Section 4. ABOUT YOU

Q36. How often do you eat Nassau grouper?
1x per week ☐, 1x per month ☐, 2x per month ☐, 3x per month ☐, other _____(please specify), never ☐

Q37. Why do you eat Nassau grouper? *Please select **top 3** reasons.*

Taste ☐
Amount of meat ☐
Cost ☐
Tradition/Culture ☐
Availability ☐

Don't like other fish ☐
Other _____ (Please specify)

Q38. Where do you purchase your fresh Nassau grouper from? *Select all that apply.*
Fishermen ☐, Fishing dock ☐ , Fish house ☐, Seafood retailer ☐, Grocery store ☐
Don't purchase fresh Nassau grouper ☐ , Only purchase Nassau grouper from Restaurants ☐

Q39. Do you fish for Nassau grouper? If no, skip to **Q44**.
Yes ☐ No ☐

Q40. How often do you fish for Nassau grouper?
1xperweek ☐, 1xpermonth ☐, 2xpermonth ☐ , 3xpermonth ☐ , other _____
(please specify)

Q41. What method(s) do you mostly use?
handline ☐ , rod-n-reel ☐ , netting ☐, traps/fish pots ☐ , Hawaiian sling/pole spear (free diving ☐,
Hawaiian sling/pole spear (hookah/compressor) ☐

Q42. How many Nassau grouper do you typically catch in one day using the method(s) selected in **Q41**?

	1-2	**3-5**	**6-10**	**>10**
Handline	☐	☐	☐	☐
Rod-n-reel	☐	☐	☐	☐
Traps/fish pots	☐	☐	☐	☐
Hawaiian sling/pole spear	☐	☐	☐	☐

Q43. Are you male ☐ or female ☐ ?

Q44. Please check the box next to your age.
18-25 ☐
26-35 ☐
36-45 ☐
46-55 ☐
56-65 ☐
66-75 ☐
>75 ☐

Q45. What is your nationality?
Bahamian ☐, Haitian ☐, American ☐ , British ☐, European ☐,
Other:_____ (please specify)

Q46. Which island do you live on? _____

Q47. What is your highest level of education?
Some high school ☐, High school diploma ☐, Technical/vocational certificate ☐,Bachelor's ☐,
Master's ☐ , PhD ☐

Q48. What organization do you work for?

_____

Definitions

Marine Protected Area (MPA) – "A clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values". IUCN

No-take MPA or reserve – A clearly defined geographical space where fishing/extractive activities are prohibited or not allowed. Similar to an MPA, no-take MPAs or reserves are "managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values". IUCN

Catch share – "a general term associated with several fisheries management strategies that dedicate a secure share of fish to individual fishermen, cooperatives, or fishing communities for their exclusive use. NOAA Fisheries

Thank you for completing this survey!

Please return it to Krista Sherman by **February 28th, 2017**.

Via

1. Email to kds204@exeter.ac.uk with 'Nassau Grouper Questionnaire' in the title.
2. Post to Krista Sherman, University of Exeter, Biosciences, Lab 201, Stocker Road, EX4 4QD, UK.

Supplementary Material 3 – Enforcement Stakeholder Questionnaire

Project Overview:

Nassau grouper are in decline globally. This iconic economically and ecologically important species is listed as endangered on the IUCN Red List and has also recently been listed as "threatened" under the United States Endangered Species Act (ESA). Management and conservation have relied heavily on biological (i.e. monitoring data) and commercial fisheries landings data to assess numbers or stock size of Nassau grouper. Population genetics offers another biological approach to more accurately assess the status of Nassau grouper in The Bahamas. I will be using both underwater visual surveys of spawning aggregations and genetic techniques to analyze the health of Nassau grouper in The Bahamas, provide estimates of the number of breeders and identify the number of distinct populations present.

This information will provide the basis for a comprehensive management strategy for Nassau grouper along with considerations of other key aspects of the species' biology and ecology. However, socioeconomic factors as well as biological factors need to be assessed to effectively manage the Nassau grouper fishery. Focus group meetings and questionnaires will be used to assess stakeholder knowledge and justify the social and economic importance of Nassau grouper to The Bahamas. Combined biological and socioeconomic data will help to guide the development of a practical, scientifically based management plan for Nassau grouper that considers the needs and perceptions of Bahamians.

Please complete and return this questionnaire to Krista Sherman of the University of Exeter. The questionnaire should take 35-40 minutes to complete. When you do this you will be supporting efforts to evaluate the status and provide recommendations to promote a sustainable Nassau grouper fishery for The Bahamas. This questionnaire helps fulfill a socioeconomic gap in our knowledge of this hugely important resource.

The researcher carrying out this project is Krista Sherman who presented her work at the Bahamas Natural History Conference on March 16th, 2016. The project aims to take biological and socioeconomic approaches to provide appropriate management measures for Nassau grouper.

Please take the time to fill in this questionnaire and return it by **February 28th, 2017**.

Via

1. Email to kds204@exeter.ac.uk with 'Nassau Grouper Questionnaire' in the title.
2. Post to Krista Sherman, University of Exeter, Biosciences, Lab 201, Stocker Road, EX4 4QD, UK.

Completion of this questionnaire is voluntary and all returned questionnaires will be coded for your anonymity.

Consent Form – Confidential Data

I understand that my participation in this project is voluntary and that I can withdraw at any time without providing a reason.

I understand that all information provided by me will remain confidential and that my name will not be directly associated with any data I provide.

I understand that I can ask for the information I provide to be deleted/destroyed at any time and that I can have access to my information.

I understand that I can omit (not answer) questions that I do not wish to answer.

I also understand that at the end of the project I will be provided with a copy of the outputs.

I, _____**(PRINT NAME)** consent to participate in this research led by Krista Sherman at the University of Exeter.

Signed: _____ Date: _____

The Bahamian Nassau Grouper Fishery

Section 1. Knowledge & Perceptions of Environmental Change

Q1. Using the scale provided, please **circle** the number that best reflects your understanding of the current state of Nassau grouper numbers (abundance) on **reefs**.

1	2	3	4	5
Significant decline	Decline	no change	Increase	Significant increase

Q2. Using the scale provided, please **circle** the number that best reflects your understanding of the current state of Nassau grouper numbers (abundance) in **nursery habitats (e.g. mangroves and seagrasses)**.

1	2	3	4	5
Significant decline	Decline	no change	Increase	Significant increase

Q3. Using the scale provided, please **circle** the number that indicates your knowledge about changes in the numbers of Nassau grouper caught (landed) in The Bahamas over the last 10 years.

1	2	3	4	5
Significant decline	Decline	no change	Increase	Significant increase

Q4. For your responses to questions 1-3, how do you know this? *Please select **all** that apply.*

	Q1	Q2	Q3	
Brochure/flyer	☐	☐	☐	
Poster	☐	☐	☐	
Environmental documentary	☐	☐	☐	
Scientific journal	☐	☐	☐	
News	☐	☐	☐	
Social Media	☐	☐	☐	
Personal observation	☐	☐	☐	
Other _____	☐ _____	☐ _____	☐ _____	(*please specify*)

Q5. Please list the top 3-5 reasons why you believe there has been a decline/increase in Nassau grouper in The Bahamas.

1. _____

2. _____

3. _____

4. _____

5. _____

Q6. Based on your understanding, please list the top 5 threats to the Nassau grouper in The Bahamas in order of importance (i.e. from 1 most important to 5 least important threat).

1. _____
2. _____
3. _____
4. _____
5. _____

Q7. On a scale from 1 (not at all concerned) to 10 (extremely concerned), please indicate your level of concern about the future sustainability of Nassau grouper in The Bahamas. *Please circle **one** number.*

1	2	3	4	5	6	7	8	9	10
Not at all concerned									Extremely concerned

Q8. Please list the top 3-5 reasons why you believe it is or is not important to conserve (sustainably manage) Nassau grouper.

1. _____

2. _____

3. _____

4. _____

5. _____

Section 2. Knowledge and Perceptions of Fishery Management in The Bahamas

Q9. Which of the following is the closed season for Nassau grouper? *Check **one** response.*

a. Dec 1 – Jan 31 ☐
b. Nov 1 – Dec 30 ☐
c. Dec 1 – Feb 28 ☐

Q10. What is the minimum size limit for fishing Nassau grouper? *Circle **one** response.*

a. 3 lb. ◯ b. 4 lb. ◯ c. 5 lb. ◯

Q11. Please rank the effectiveness of the following current methods for managing Nassau grouper (1=Very ineffective; 2=Ineffective; 3=Neither effective nor ineffective; 4=Effective; 5=Very effective; 6=Don't know). *Check the **one** answer for each method.*

	1	**2**	**3**	**4**	**5**	**6**
I. Minimum size limit	☐	☐	☐	☐	☐	☐
II. Closed season	☐	☐	☐	☐	☐	☐
III. Marine Protected Areas (MPAs)	☐	☐	☐	☐	☐	☐
IV. No-take MPAs or reserves	☐	☐	☐	☐	☐	☐

Q12. For each of your responses to **Q11**, please explain why you consider these methods as effective or not effective.

I.

II.

III.

IV.

Q13. Use the scale below to reflect how the current methods for managing Nassau grouper affect Bahamian fishers. 1=Very negative; 2=Negative; 3=Neither negative nor positive; 4=Positive; 5=Very positive; 6 = Don't know. *Check the **one** answer for each method.*

	1	**2**	**3**	**4**	**5**	**6**
I. Minimum size limit	☐	☐	☐	☐	☐	☐
II. Closed season	☐	☐	☐	☐	☐	☐
III. Marine Protected Areas (MPAs)	☐	☐	☐	☐	☐	☐
IV. No-take MPAs or reserves	☐	☐	☐	☐	☐	☐

Q14. Explain your responses in **Q13**. How do Nassau grouper fisheries regulations affect Bahamian fishermen?

I.

II.

III.

IV.

Q15. Please list the 3 main barriers to effective management of the Nassau grouper fishery in The Bahamas. Rank your top 3 responses (from 1 most important to 3 least important barriers).

1. _____

2. _____

3. _____

Q16. For each main barrier you listed in **Q15**, provide **one** way in which that barrier can be addressed (corrected/changed) to more effectively manage the Nassau grouper fishery in The Bahamas.

1. _____

2. _____

3. _____

Q17. In your opinion, what is the best method/strategy to improve sustainability of the Bahamian Nassau grouper fishery? *Please explain your answer.*

Q18 a. Would you support changes to existing regulations and **b.** the creation of new regulations to promote recovery of the Nassau grouper fishery?

a. Yes ☐ No ☐ **b.** Yes ☐ No ☐

Q19. Choose the amendments or changes to existing regulations that you would be willing to support. *Select **all** that apply.*

Increased minimum size limit ☐
Extended closed season ☐
New Marine protected areas (MPAs) ☐
New no-take MPAs or reserves ☐

Q20. Which of the following new regulations would you be willing to support? *Select **all** that apply.*

Establish quotas/bag limits ☐
Protection of fish spawning aggregation sites (FSAs) ☐
Ban use of hookahs (compressor diving) ☐

Ban use of traps/fish pots at FSAs during the closed season ☐
Establish maximum size limit ☐
New No-take MPAs or reserves ☐
Temporary ban (e.g. 10 yrs.) on fishing Nassau grouper ☐
Catch shares for fishers ☐
Other _____ (Please specify)

Q21. Do you have any other recommendations that would improve fisheries management for Nassau grouper? **(Max 100 words)**

Section 3. Science, Education & Outreach

Q22. Please list 3-4 current approaches/practices (i.e. within the last 5 yrs.) being used to help improve outreach to the **a.** fishing sector and **b.** general public for Nassau grouper management?

a. Fishing Sector

1. _____

2. _____

3. _____

4. _____

b. General Public

1. _____

2. _____

3. _____

4. _____

Q23. Please prioritize and rate the effectiveness (1=Very ineffective; 2= Ineffective; 3= Neither effective nor ineffective; 4= Effective; 5= Very effective) of approaches/practices you listed in **Q22** for a. the fishing sector and b. general public.

a. Fishing Sector

Approaches	1	2	3	4	5
1.	☐	☐	☐	☐	☐
2.	☐	☐	☐	☐	☐
3.	☐	☐	☐	☐	☐
4.	☐	☐	☐	☐	☐

b. General Public

Approaches	1	2	3	4	5
1.					
2.					
3.					
4.					

Q24. What new approaches can be used to improve management of Nassau grouper with a view to increase knowledge, change attitudes/perceptions and influence behaviour of fishers and the public? **(Max 100 words)**

Q25. How will you know if these approaches worked? **(Max 100 words)**

Q26. How quickly should these changes be implemented? *Select **one** response.*

Immediately
Within 6 mo
Within 1 yr
Within 2-5 yrs
Within 6-10 yrs

Q27. How can your organization improve upon the support provided to fishers, policy-makers, law enforcement, scientists, marine resource managers and other organizations? **(Max 100 words)**

Q28. What is the best way for information on Nassau grouper research to be communicated to your organizations?

Report(s) ☐, Popular article(s) ☐, Peer-reviewed manuscript(s) ☐, Public presentation(s) ☐
Social media ☐, Other _____ (please specify)

Q29. What additional/new information would you need to support changes to existing regulations or the creation of new regulations?

Q30. Is there an area of Nassau grouper research that is particularly important? *Select **all** applicable answers.*

Spawning aggregations ☐
Population structure and connectivity ☐
Stock assessments ☐
Migration patterns ☐
Socioeconomic evaluation of the fishery ☐
Impacts of threats ☐
Larval behaviour/ecology ☐
Other: _____ (Please specify)

Q31. Please respond to the following statements (1 =strongly agree; 5=strongly disagree; 6=don't know). *Select **one** response for each statement.*

	1	2	3	4	5	6
1. I am unaware of scientific research on Nassau grouper.						
2. Scientific research on Nassau grouper is unnecessary.						
3. Genetics is a useful tool to assess numbers of Nassau grouper.						
4. Monitoring fish spawning aggregations is unnecessary.						
5. Scientists communicate their results effectively.						
6. I do not trust scientific information/data.						
7. Science should be used to inform management.						

Section 4. Legislation & Enforcement

Q32. Please rate the following potential drivers/causes for illegal fishing (i.e. catching undersized fish or fishing during the closed season) from 1=Most important to 5=Least important.

	1	2	3	4	5
Poverty					
Access rights to fishing grounds					
Consumer demand					
Lack of knowledge of fishery regulations					
Lack of economic alternatives					
Population growth					
Foreign recreational fishers					
Foreign commercial fishers					
Corruption/bribery					

Q33. Use the scale provided to select **one** response reflecting how effective enforcement is for managing Nassau grouper. (1=Very ineffective; 2=Ineffective; 3=Neither effective nor ineffective; 4=Effective; 5=Very effective; 6=Not applicable). *Select **one** response for each scenario.*

	1	2	3	4	5	6
Water-based patrols (e.g. patrol boat)						
Aerial patrols (e.g. airplane, drone)						
Fishery landings site inspections						
At first point of sale (e.g. public docks, fish ramps)						
At consumer level (e.g. seafood retailer and restaurant inspections)						

Q34. Based on your knowledge, please **select all the organizations** that are involved in making decisions about Nassau grouper management?

- Department of Marine Resources ☐
- Royal Bahamas Defence Force ☐
- Customs ☐
- Immigration ☐
- Bahamas Commercial Fishers Alliance ☐

National Non-governmental organizations (NGOs) (e. g. BNT) ☐
International NGOs (e.g. TNC) ☐
Research Institutions (e.g. CEI) ☐
Other _____ ☐

Q35. Based on your knowledge, which agencies are **legally mandated** to enforce fisheries regulations pertaining to Nassau grouper? *Select **all** that apply.*

Department of Marine Resources ☐
Royal Bahamas Defence Force ☐
Customs ☐
Immigration ☐
Bahamas Commercial Fishers Alliance ☐
National Non-governmental organizations (NGOs) (e. g. BNT) ☐
International NGOs (e.g. TNC) ☐
Research Institutions (e.g. CEI) ☐
Other _____ ☐

Q36. Please list the **enforcement agencies** which have training programmes for their officers that provide information on Bahamian fisheries regulations, locations and rules of MPAs and no-take marine reserves.

Q37. How many times per year is the training programme delivered?
1x per year ☐ 2x per year ☐ every 2 years ☐ other ☐ unknown ☐

Q38. If training programmes do not exist, do you believe that developing one would be useful?
Yes ☐ No ☐

Q39. What resources/materials would be useful to support enforcement officer training? *Select your **top 3** responses.*

Training manual ☐
Video ☐
Podcast ☐
Webinar ☐
Workshop/presentation ☐
Infographic ☐
Brochure ☐

Q40. Are the current available resources sufficient to enforce fishery & MPA regulations?

Yes ☐ No ☐ Don't know ☐

Q41. What are the top 3 difficulties your respective organizations face with enforcing fisheries regulations?

1. _____

2. _____

3. _____

Q42. Have there been any attempts to resolve these issues and if so what was the outcome? **(Max 100 words)**

Q43. What additional/new information would you require to better enforce fisheries regulations and how should this information be provided?

Q44. How can policy-makers, scientists, marine resource managers, etc. help to better support you in your role as law enforcement officers? **(Max 100 words)**

Q45. How many times per year are reports of illegal fishing (i.e. catching undersized fish or fishing during the closed season) for Nassau grouper by **Bahamians** recorded?

0 ☐ 1-5 ☐ 6-10 ☐ 11-20 ☐ >20 ☐

Q46. How many times per year are reports of illegal fishing (i.e. catching undersized fish or fishing during the closed season) for Nassau grouper by **non-Bahamians (foreigners)** recorded?

0 ☐ 1-5 ☐ 6-10 ☐ 11-20 ☐ >20 ☐

Q47. On an annual basis, how many violators of Nassau grouper fisheries regulations are fined?

0 ☐ 1-5 ☐ 6-10 ☐ 11-20 ☐ > 20 ☐

Q48. How many people are arrested annually for violating Nassau grouper fishery regulations?

0 ☐ 1-5 ☐ 6-10 ☐ 11-20 ☐ > 20 ☐

Q49. How many of the people arrested annually are **Bahamian**? _____

Q50. How many of the people arrested annually are **non-Bahamian**? _____

Q51. Penalties for violators of Nassau grouper fishery regulations: (1 = are satisfactory; 2 = should increase; 3= should decrease; 4=don't know). *Select the answer that best reflects your opinion.*

1 ☐ 2 ☐ 3 ☐ 4 ☐

Q52. From an enforcement perspective, do you have any new recommendations that would improve fisheries management for Nassau grouper?

Q53. In your opinion, which organizations *should* be **managing** the fishery? *Select **all** that apply.*

Department of Marine Resources	☐
Royal Bahamas Defence Force	☐
Royal Bahamas Police Force	☐
Customs	☐
Immigration	☐
Bahamas Commercial Fishers Alliance	☐
National Non-governmental organizations (NGOs – e.g. BNT)	☐
International NGOs	☐
Research Institutions	☐

Q54. In your opinion, which organizations *should* be **enforcing** fishery regulations? *Select **all** that apply.*

- ☐ Department of Marine Resources
- ☐ Royal Bahamas Defence Force
- ☐ Royal Bahamas Police Force
- ☐ Customs
- ☐ Immigration
- ☐ Bahamas Commercial Fishers Alliance
- ☐ National Non-governmental organizations (NGOs – e. g. BREEF)
- ☐ International NGOs (e.g. TNC)
- ☐ Research Institutions (e.g. CEI)

Q55. *Please rank the following **organizations** in terms of whether or not they should have a say in management decisions?? 1-6 (1 should have the most say) or 0 (no say).*

Department of Marine Resources	Select
Royal Bahamas Defence Force	Select
Royal Bahamas Police Force	Select
Bahamas National Trust	Select
Customs	Select
Immigration	Select
Bahamas Commercial Fishers Alliance	Select

Q56. What role can law enforcement agencies play to help provide support (e.g. logistical, financial, etc.) for on-going research, monitoring, outreach and advocacy? **(Max 100 words)**

Q57. Is there anything else you would like to say about managing Nassau grouper in The Bahamas? **(Max 150 words)**

Q58. How often do you eat Nassau grouper?
1x per week ☐, 1x per month, ☐ 2x per month, ☐ 3x per month, ☐ other _____(please specify), never ☐

Q59. Why do you eat Nassau grouper? *Please select **top 3** reasons.*

Taste ☐
Amount of meat ☐
Cost ☐
Tradition/Culture ☐
Availability ☐
Don't like other fish ☐
Other _____ (Please specify)

Q60. Where do you purchase your fresh Nassau grouper from? *Select all that apply.*
Fishermen ☐, Fishing dock ☐, Fish house ☐, Seafood retailer ☐, Grocery store ☐
Don't purchase fresh Nassau grouper ☐, Only purchase Nassau grouper from Restaurants ☐

Q61. Do you fish for Nassau grouper? If no, skip to **Q65**.
Yes ☐ No ☐

Q62. How often do you fish for Nassau grouper?
1x per week ☐ 1x per month ☐ 2x per month ☐ 3x per month ☐ other _____ (please specify)

Q63. What method(s) do you mostly use?
handline ☐ rod-n-reel ☐ netting ☐ traps/fish pots ☐ Hawaiian sling/pole spear (free diving) ☐
Hawaiian sling/pole spear (hookah/compressor) ☐

Q64. How many Nassau grouper do you typically catch in one day using the method(s) selected in **Q63**?

	1-2	**3-5**	**6-10**	**>10**
Handline	☐	☐	☐	☐
Rod-n-reel	☐	☐	☐	☐
Traps/fish pots	☐	☐	☐	☐
Hawaiian sling/pole spear	☐	☐	☐	☐

Q65. Are you male ☐ or female ☐?

Q66. Please check the box next to your age.
18-25 ☐
26-35 ☐
36-45 ☐
46-55 ☐
56-65 ☐
66-75 ☐
>75 ☐

Q67. What is your nationality?
Bahamian ☐ Haitian ☐ American ☐ British ☐ European ☐
Other:_____ (please specify)

Q68. Which island do you live on? _____

Q69. What is your highest level of education?
Some high school ☐ High school diploma ☐ Technical/vocational certificate ☐ Bachelor's ☐ Master's ☐
PhD ☐

Q70. What organization do you work for?

_____

Definitions

Marine Protected Area (MPA) – "A clearly defined geographical space, recognised, dedicated and managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values". IUCN

No-take MPA or reserve – A clearly defined geographical space where fishing/extractive activities are prohibited or not allowed. Similar to an MPA, no-take MPAs or reserves are "managed, through legal or other effective means, to achieve the long-term conservation of nature with associated ecosystem services and cultural values". IUCN

Catch share – "a general term associated with several fisheries management strategies that dedicate a secure share of fish to individual fishermen, cooperatives, or fishing communities for their exclusive use. NOAA Fisheries

Thank you for completing this survey!

Please return it to Krista Sherman by **February 28th, 2017**.

Via

1. Email to kds204@exeter.ac.uk with 'Nassau Grouper Questionnaire' in the title.
2. Post to Krista Sherman, University of Exeter, Biosciences, Lab 201, Stocker Road, EX4 4QD, UK.

Chapter VI

Contemporary and emerging fisheries in The Bahamas – conservation and management challenges, achievements and future directions

Manuscript in press: Fisheries Management and Ecology

Authors: Sherman KD, Shultz AD, Dahlgren CP, Thomas C, Brooks E, Brooks A, Brumbaugh DR, Gittens L, Murchie KJ

Contemporary and emerging fisheries in The Bahamas – conservation and management challenges, achievements and future directions

Krista D. Sherman[1,2,3*], Aaron D. Shultz[4,5,6] Craig P. Dahlgren[2,7], Claire Thomas[8], Edward Brooks[9], Annabelle Brooks[9], Daniel R. Brumbaugh[10], Lester Gittens[11], Karen J. Murchie[3]

[1]University of Exeter, Department of Biosciences, College of Life and Environmental Sciences, Stocker Road, Exeter, EX4 4QD, United Kingdom
[2]Science and Policy, Bahamas National Trust, P.O. Box N-4105, Nassau, Bahamas
[3]Daniel P. Haerther Center for Conservation and Research, John G. Shedd Aquarium, 1200 South Lake Shore Drive, Chicago, Illinois, USA, 60605
[4]Great Lakes Indian Fish and Wildlife Commission, 72682 Maple St., Odanah, Wisconsin, USA, 54861
[5]Fisheries Conservation Foundation, 302 E. Green Street #2102, Champaign, Illinois, USA, 61825
[6]Michigan State University, Department of Fisheries and Wildlife, Natural Resources Building, 480 Wilson Road, Room 13, East Lansing, Michigan 48824
[7]Perry Institute for Marine Science, P. O. Box 435 Waitsfield, VT 05673, USA
[8]College of DuPage, 425 Fawell Blvd, Glen Ellyn, IL 60137, USA
[9]Cape Eleuthera Institute, P. O. Box EL-26029, Eleuthera, Bahamas
[10]University of California Santa Cruz, Institute of Marine Sciences 1156 High Street, Santa Cruz, CA, USA, 95064
[11]Department of Marine Resources, P. O. Box N-3028, Nassau, Bahamas

*Corresponding Author Contact Information
Email: kds204@exeter.ac.uk or krista.daniellesherman@gmail.com
Tel: +44 (0) 1392 724677.

Running Head: Contemporary and emerging fisheries in The Bahamas

Abstract

The harvest of marine resources has long-standing cultural and economic importance to The Bahamas and other small island developing states. Tourists and residents place a demand on local marine resources, particularly Caribbean spiny lobster, *Panulirus argus* (Latreille), queen conch, *Lobatus gigas* (Linnaeus) and Nassau grouper, *Epinephelus striatus* (Bloch) and many fishery products are also sold on the global market. Illegal, unreported and unregulated fishing coupled with inadequate regulations and enforcement are the main factors contributing to the decline of Bahamian fisheries along with other anthropogenic impacts. This paper reviews the status of fisheries management in The Bahamas using economically and ecologically important species as case studies to highlight conservation successes, knowledge gaps and deficiencies in existing management approaches. The review concludes with an examination of how emerging fisheries and improved conservation management strategies have the potential to improve economic and food security throughout the archipelago.

Key Words: commercial fisheries; extractive fisheries; marine protected areas; recreational fishing; small island developing states; sustainable fisheries management

1.0 Introduction

Worldwide, fisheries are in decline by approximately 1.2 million tonnes per year (Pauly & Zeller, 2016) with an estimated 31 % of marine fish species overfished (FAO, 2016). The Fisheries and Agriculture Organization (FAO) of the United Nations estimates that 56.6 million people are employed through the fisheries and aquaculture sector (FAO, 2016). However, anthropogenic impacts including overfishing, invasive species, climate change, coastal development and pollution are significant threats to biodiversity, ecosystem resilience and socioeconomic stability (Hoegh-Guldberg & Bruno, 2010; Cheung et al., 2012; Albins & Hixon, 2013). Although the status of fish stocks and the rate at which declines are occurring can be debated, there is agreement that declines are likely to persist without strategic management (e.g., Worm et al., 2009; Branch et al., 2011). Small island developing states (SIDS), such as The Bahamas, are vulnerable to the impacts listed above and have experienced declining trends

for many commercially important species (e.g. Stallings, 2009; Stoner et al., 2012b,c; Sherman et al., 2016). Managing fisheries in SIDS is particularly challenging due to the combined effects of limited means for monitoring and enforcement, the strong socio-cultural and economic drivers associated with harvesting resources for local consumption and export and the highly complex and dynamic nature of the marine ecosystems and governance frameworks under which they exist (FAO, 1999; Douglas, 2006).

Fisheries and marine resource management in The Bahamas is further complicated by its broad spatial scale. The country consists of 700 relatively flat islands and 3,000 cays encompassing ~300,000 km^2 of land and sea (Buchan, 2000; Fig.1). The islands of New Providence and Grand Bahama are the most developed and heavily populated with approximate population sizes of 250,000 and 50,000, respectively (Mackey et al., 2010). The remaining inhabited islands, referred to as Family Islands, are more remote with limited infrastructure and smaller population sizes (range 72 – 17,224 people; Mackey et al., 2010).

As of 2010, at least 1 % of the surveyed population (351,461 people) directly earned a living through commercial fisheries in The Bahamas (Anon, 2010; Anon, 2012). While tourism is the primary industry, contributing 43.6 % of the total gross domestic product (GDP) in 2014 to The Bahamas (Anon, 2014), fisheries are inherently connected to the industry with demands for local fish protein from both tourists and residents alike (Smith & Zeller, 2013). In addition to supporting the tourism industry, commercial fishing contributes to 2 % of the GDP (Anon, 2014). Subsistence fishing for personal food security is undocumented but is critically important, particularly in the Family Islands. Given the importance of the fisheries sector to The Bahamas, and the current movement of the United Nations sustainable development goals (http://www.un.org/sustainabledevelopment/), this review aims to 1) provide a timely synthesis and assessment of the main contemporary extractive fisheries in terms of stock status and management challenges, 2) examine emerging fisheries and their implications for potential economic and food security and 3) highlight conservation and management strategies for species that influence ecosystems and the economy in The Bahamas.

1.2 Contemporary fisheries in The Bahamas

Contemporary Bahamian fisheries are comprised of commercial, sport, recreational and subsistence fishing, with most of the reported revenue generated from commercial fishing (Smith & Zeller, 2013). Commercial fishing typically occurs on Great Bahama, Little Bahama and Cay Sal banks and is legally restricted to vessels owned by Bahamians (Anon 2008; Fig. 1). Commercial fisheries data are collected via sampling at a proportion of frequently used landing sites and from purchasing reports from seafood processing companies (Smith & Zeller, 2013). Main landing sites in The Bahamas are located on the islands of New Providence, Eleuthera, Grand Bahama, Abaco and Long Island (FAO, 2009), although fishing occurs throughout the country. Data on recreational and subsistence fisheries sectors are not routinely collected due to limited resources. Sport fishery data are occasionally collected during tournaments, but the quality and accuracy of these data are variable (Smith & Zeller, 2013). However, in a recent economic valuation, recreational and sport fisheries have been reported to generate approximately US $527 million per annum (Maycock, 2016). Since 1980, the Department of Marine Resources (DMR) has produced annual reports based on data collated from the country.

All fisheries are primarily managed by the Government of The Bahamas through the DMR, which is responsible for the sustainable use of fisheries resources for the benefit of the Bahamian people. The Fisheries Resources (Jurisdiction and Conservation) Act 1977 (hereafter referred to as Fisheries Act) is the legislative framework governing the DMR and provides specific details on fisheries rules and regulations that can be enforced by DMR fisheries officers, and officers of the Royal Bahamas Defence Force (RBDF), Royal Bahamas Police Force and Customs (http://laws.bahamas.gov.bs/cms/images/LEGISLATION/PRINCIPAL/1977/1977-0013/FisheriesResourcesJurisdictionandConservationAct_1.pdf). Wardens from the Bahamas National Trust (BNT) also assist with fisheries management by enforcing regulations within the Bahamas National Protected Area System (BNPAS).

The main species currently targeted for commercial and subsistence fisheries include Caribbean spiny lobster, *Panulirus argus* (Latreille), queen conch, *Lobatus* (formerly *Strombus*) *gigas* (Linnaeus), and medium- to large-bodied reef fish, including Nassau grouper, *Epinephelus striatus* (Bloch), other

groupers (Epinephelidae), snappers (Lutjanidae), grunts (Haemulidae) and jacks (Carangidae) as well as stone crab, *Menippe mercenaria* (Say) (BDF, 1986; FAO, 2009; Supplementary Table 1). Recreational fisheries primarily target bonefish, *Albula vulpes* (Linnaeus), permit, *Trachinotus falcatus* (Linnaeus), tarpon, *Megalops atlanticus* Valenciennes, great barracuda *Sphyraena barracuda* (Edwards), larger demersal (e.g., black grouper, *Mycteroperca bonaci* (Poey)) and pelagic species (e.g., dolphinfish, *Coryphaena hippurus* Linnaeus, and wahoo, *Acanthocybium solandri* (Cuvier)). The most commonly used fishing methods include condominiums, also called condos or casitas (flat structures that attract lobsters) and wooden lathe traps [specifically for Caribbean spiny lobster], traps or fish pots, Hawaiian slings, pole spears, line fishing with hand-line or rod and reel and nets. For commercial fishing, only gill nets, drag nets, cast nets and seine nets with a minimum mesh size of 50.8 mm can be used with limited exceptions, e.g., if harvesting herring (Clupeidae) or silversides (Atherinidae). Of the commercial and subsistence species, snappers, grunts, jacks, and black grouper are currently unmanaged through specific fishery regulations such as size limits or closed seasons. (Supplementary Table 2). For recreational fisheries, foreign vessels are assigned bag limits, which restrict harvest to 9.07 kg of scalefish, 6 conch and 10 spiny lobster per vessel, at any time. However, for all marine species harvesting on SCUBA and the use of spear-guns is prohibited throughout the country.

Of all fishery species, Caribbean spiny lobster, queen conch and Nassau grouper are monetarily the most valuable species, generating over US $1.4 billion per annum in combined commercial landings for The Bahamas over the past two decades (Supplementary Table 1) but are at risk of overexploitation. Current management practices and monitoring efforts of the three most valuable species in The Bahamas are described below, followed by a discussion of how these fisheries could become sustainable through additional management actions.

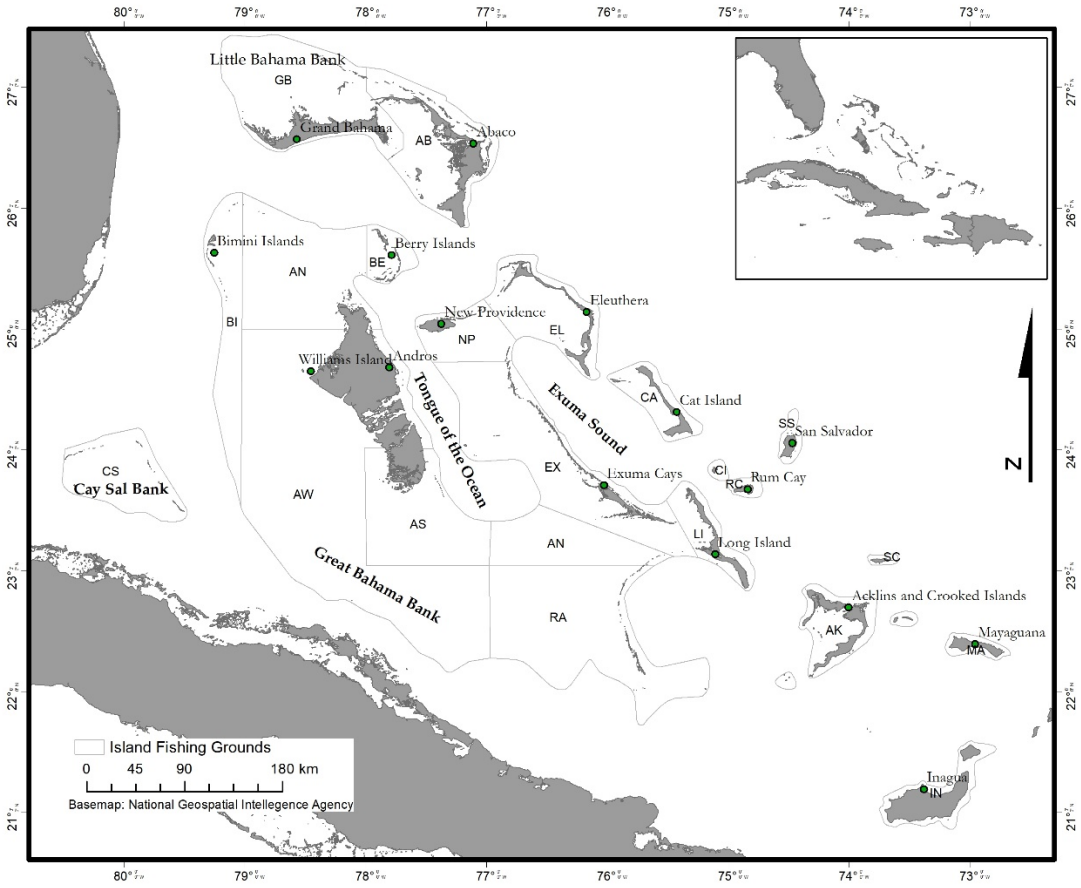


Figure 1. Map of The Bahamas showing major fishing islands and fishing banks.

2. Species at risk

2.1. *Caribbean Spiny Lobster*

The Caribbean spiny lobster represents the mainstay of the commercial fishing industry in the archipelago (FAO, 2009). From 1995 to 2015, Caribbean spiny lobster represented 80–90 % of the total value of the fisheries landings. The majority (>90 %) of landed Caribbean spiny lobsters are exported as tails (FAO, 2009), with The Bahamas making up 13 % of the Caribbean spiny lobster imports to the United States, second only to Brazil at 22 % (Sullivan, 2013). Due to the volume of landings, the high value (Supplementary Table 1), the number of fishers employed (approximately 9,300 individuals; FAO, 2009) and the use of vessels that can operate up to four weeks at sea, the Caribbean spiny lobster

fishery is the only truly large-scale commercial fishery in The Bahamas (Smith & Zeller, 2013).

The IUCN Red List designation for the Caribbean spiny lobster is “data deficient” (Butler et al., 2011; Supplementary Table 2) and for The Bahamas the status of the stock has been unknown due to uncertainty in the length-converted-catch-curve estimates and estimated catch per unit effort (CPUE) provided by DMR (CRFM, 2008). Since 2010, The Bahamas Caribbean spiny lobster fishery has been in the process of receiving Marine Stewardship Council (MSC) certification to ensure the long-term sustainability of the fishery (WWF, 2013). To this end, DMR has requested that processors and fishers provide data on lobster tail weight, fishing location, gear used and by-catch to improve stock assessment, harvest control rules and reference points (MRAG Ltd., 2015).

Another critical issue challenging the Caribbean spiny lobster fishery is the government’s capacity to address illegal, unreported and unregulated (IUU) fishing (CRFM, 2014). Approximately 36 % of all Bahamian landings fall under the IUU category (Medley & Gittens, 2012) and most of this product enters the Dominican Republic, representing a major challenge for sustainable fisheries management. To combat local IUU fishing practices, local non-government organizations (NGOs) have been educating both fishers and the public about minimum size limits and other fishery regulations (MRAG Ltd., 2015). The Caribbean Regional Fisheries Mechanism (CRFM) members, including The Bahamas, are considering a common closed season (i.e., countries share the same closed season) for neighbouring countries to discourage foreign illegal fishing) of Caribbean spiny lobsters (CRFM, 2014).

Due to the high reproductive potential of the Caribbean spiny lobster (Ehrhardt, 2005) and the relatively low-tech gear types used (Callwood, 2010), the Caribbean spiny lobster fishery in The Bahamas is poised to be a sustainable fishery if the challenges stated above can be addressed. However, new scientific data regarding population connectivity and potential for self-recruitment (proportion of larvae returning to their natal population; e.g., Callwood, 2010; Kough et al., 2013, but see Naro-Maciel et al., 2011) may have direct management implications (e.g. Lipcius et al., 2013), further highlighting the need for a comprehensive stock assessment combined with spatial analysis of fishing effort (Callwood, 2016). High harvest of Caribbean spiny lobsters may

have significant impacts on reef ecosystems because of their important ecological roles on reefs (e.g., Boudreau & Worm, 2012). Similarly, fishing gears like condos can affect benthic communities and patch reef dynamics (e.g., Mintz et al., 1994). As such, understanding the ecosystem impacts of high harvest of lobsters and use of fishing gears on benthic community structure are essential for ecosystem-based management efforts in The Bahamas.

2.2. *Queen Conch*

Queen conch have been a significant part of The Bahamas fishery since the time of the Lucayans (900–1,500 AD) and remain the most important fishery species as a dietary staple and cultural icon. Queen conch are also economically important, constituting the second biggest fishery in the country, with landings valued between US $3–5 million per year (Supplementary Table 1). A total of 182,271 kg in queen conch meat and products generated over US $2.4 million in exports in 2015 (DMR unpubl. data). Queen conch are generally harvested by free diving, or with an air compressor (restricted for use within depths of 9.1–18.3 m), which requires a licence and SCUBA certification (Fisheries Act; Supplementary Table 2). Regulations include a ban on fishing queen conch without the shell having a fully formed flared lip, a ban on use of SCUBA and an export quota (Supplementary Table 2).

Queen conch are important grazers in seagrass and macroalgal communities, which in turn contribute to the health of coral reef systems (Lapointe et al., 2004). They exhibit slow growth and maturation, attaining sexual maturity at ~ 6 years old and a shell lip thickness of 15 mm (Stoner et al., 2012a). They also exhibit density-dependent reproduction, with a minimum density of 50 to 75 adult queen conch/ha required for reproduction (Stoner et al., 2012b). Because of their life history characteristics, high market demand and unsustainable harvesting, conch populations are threatened throughout their range (Theile, 2005). As a result, queen conch have been listed on Appendix II of Convention on the International Trade of Endangered Species (CITES) since 1992 (https://www.cites.org/eng/prog/queen_conch). This appendix lists species that might be threatened with extinction, so trade of these species must be regulated to ensure their survival.

A few management strategies have been implemented to protect the queen conch fishery. The first strategy includes limiting harvest to individuals with a flared shell lip. However, there is ambiguity in stakeholder interpretation of this feature. Research has shown that using the flared lip guideline as a management tool allows for legal harvest of juvenile queen conch, which impedes stock replenishment (Clark et al. 2005). More recently, researchers and conservationists have advocated for amending the fisheries regulation to prevent harvesting of immature queen conch with <15 mm lip thickness (Stoner et al., 2012a; www.bnt.bs/science/conchservation). No-take marine protected areas (MPAs) have also been a partially effective management strategy for queen conch. The Exuma Cays Land and Sea Park (ECLSP) had historically healthy adult queen conch populations, but repeated surveys have shown that deep-water populations inside the no-take MPA are sharply declining and the overall population is aging with little signs of recruitment (Kough et al. 2017). However, conch densities inside the ECLSP still surpass those outside the MPA (Stoner et al., 2012c).

A multi-partner, multi-sector 'Conchservation' campaign was launched in 2013 in The Bahamas with the goal of promoting a sustainable queen conch fishery. Objectives of the campaign are to increase public awareness about the status of queen conch stocks, update legislation to reflect the best science available and incorporate co-management strategies for queen conch fisheries (www.bnt.bs/science/conchservation). In addition to education and legislative efforts, more comprehensive stock assessments are also needed. Although assessments have been completed in several queen conch fishing grounds (Stoner et al., 2009; Stoner et al., 2013; Thomas et al., 2015), large areas of the country have not been surveyed that are vulnerable to overfishing (Stoner et al., 2015). Since density is an important consideration for management, with a goal of maintaining minimum densities of 100 adult queen conch/ha (Stoner et al., 2013) these surveys are critically needed. Future research should also include identifying high quality habitat and better understanding source-sink dynamics (Kough et al., 2017) to inform the placement of MPAs.

2.3. *Nassau grouper*

Nassau grouper, widely dispersed among insular marine habitats (Sadovy & Eklund, 1999), are normally solitary, but migrate long distances seasonally to reproduce at transient fish spawning aggregations (FSAs) in synchrony with the lunar cycle (Dahlgren et al., 2016a). High catchability during the annual reproductive season at spatially predictable FSAs combined with slow growth and sexual maturity have led to significant declines (~ 60%) in global Nassau grouper populations (Sadovy de Mitcheson and Colin, 2012). Consequently, Nassau grouper haven been re-classified by the IUCN as critically endangered (Supplementary Table 2) and were officially listed as threatened under the United States Endangered Species Act in June 2016 (Carpenter et al., 2015; Federal Register, 2016).

Like queen conch, Nassau grouper are iconic species and a staple of the Bahamian diet, providing income for thousands of fishers through a commercial fishery (Cushion & Sullivan-Sealey, 2008). Sherman et al. (2016) report that the average revenue generated from the commercial Nassau grouper fishery exceeds US $1 million per year. Over the last 20 years, a total of 4,698,310 kg of Nassau grouper, valued at more than US $32.5 million, have been landed in The Bahamas (Supplementary Table 1). However, the overall economic contribution to the country remains unquantified because income derived from subsistence and recreational fisheries has not been evaluated.

Overfishing and subsequent FSA collapses have been reported throughout the native range of the species (Sala et al., 2001; Stump et al., 2017). Drastic reductions in Nassau grouper abundance are likely to negatively impact the long-term survivability of the species and overall reef health (Sadovy de Mitcheson and Colin, 2012). Compared with the Caribbean, densities and sighting frequencies of Nassau grouper in The Bahamas are relatively high (Stallings, 2009; Dahlgren et al., 2016b). This may be due to availability of required habitats or the occurrence of a greater number of reported Nassau grouper FSAs in the country. However, an analysis of long-term fishery-independent underwater visual survey data collected over 14 years shows significant declines in Nassau grouper densities throughout The Bahamas (Dahlgren et al., 2016b; Marks & Laing, 2016).

These declines have occurred despite Nassau grouper having received some level of harvest restriction in The Bahamas for the past three decades (Sherman et al., 2016; Supplementary Table 2). A minimum size limit (≥1.36 kg)

and seasonal closures for Nassau grouper during several months when FSAs occur began in 2004 but varied annually in timing until the Fisheries Act was amended in 2015 to include a fixed closed season (Supplementary Table 2). Overfishing, inadequate enforcement and annual variability in the length and timing of fisheries regulations have contributed to declines in abundance of 70 % or more (Cheung et al., 2013) with predictions of extinction due to overexploitation (Sadovy de Mitcheson et al., 2013). Sherman et al., (2016) reported that commercial landings of Nassau grouper have declined by 86 % throughout the country, with 20–40 % of reported landings caught illegally during the closed season, highlighting the need for a more strategic approach to conservation management for the species. Evidence that most fish of the minimum size are immature (Sadovy & Colin, 1995) and do not make spawning migrations (Dahlgren et al., 2016a) also suggests that a larger minimum size is needed. Management recommendations for Nassau grouper have been outlined by Sherman et al. (2016), and a national conservation management plan is being developed to facilitate population recovery (Sherman et al., 2018). Conservation of Nassau grouper is dependent on the timely implementation of science-based recommendations by policy makers and a shift in public attitudes and perceptions regarding compliance for national fishery regulations and ongoing conservations efforts (Sherman et al., 2016).

3.0. Achievements in species conservation

While improved management of the Caribbean spiny lobster, queen conch and Nassau grouper fisheries is advocated, it is also valuable to highlight promising steps in the management of other Bahamian fisheries. Here, achievements in conservation for a variety of shark, sea turtle and bonefish species are discussed, along with future suggestions for their management.

3.1. *Shark conservation in The Bahamas*

The Bahamas are home to a diverse and abundant elasmobranch assemblage which supports the largest shark-diving industry in the world, estimated to contribute US $113.8 million annually to the local economy (Haas et al., 2017). Historically, commercial shark fisheries in The Bahamas have

been limited. The first recorded commercial harvest of sharks in The Bahamas was reported in 1993 when 37 metric tonnes were declared to the Food and Agriculture Organisation of the United Nations (FAO FISHSTAT, 1950–2015), coinciding with the emergence of a nascent and completely unregulated longline fishery. In response to concerns regarding the sustainability of this new fishery, a ban of commercial longlines was declared in December 1993 (Burgess & Fordham, 2005). This ban outlawed the most economically viable method of commercial shark capture and subsequently the reported catches of sharks fell to 5, 3, 2 and 1 metric tonnes from 1996–1999, respectively. In the absence of a viable commercial shark fishery and recognising the financial importance of shark related tourism, the Bahamian government further strengthened its protective regulations in 2011, declaring Bahamian waters a shark sanctuary and prohibiting the harvest of sharks by any capture method throughout the 654,715 km^2 Bahamian EEZ. This action was the result of a significant public relations campaign led by the BNT and the Pew Environment Group. The combination of the 1993 longline ban and the 2011 establishment of the shark sanctuary have ensured that there has been virtually no commercial harvest of elasmobranchs within the Bahamian EEZ.

These management decisions have been effective at protecting coastal species with limited home ranges (e.g. the reef shark, *Carcharhinus perezi* (Poey); Shipley et al., 2017), which exhibited no long-term (1979–2013) decline in abundance (Edward Brooks, Cape Eleuthera Institute, unpubl. data). Conversely, the abundance of transboundary and highly migratory species with pelagic components to their life history, for example the tiger shark, *Galeocerdo cuvier* (Péron & Lesueur), which is known to move seasonally between North Atlantic and Bahamian waters (Lea et al., 2015), declined 22 % in the same period (Edward Brooks, Cape Eleuthera Institute, unpubl. data).

The continued exploitation of highly migratory species has implications for the economy of several Bahamian Family Islands. In particular, “rare-species” dives that focus on interactions with highly migratory charismatic species in specific locations at specific times of year are at risk: for example, oceanic whitetip shark, *Carcharhinus longimanus* (Poey) dives in southern Cat Island (Howey-Jordan et al., 2013), great hammerhead shark, *Sphyrna mokarran* (Rüppell) dives in South Bimini (Guttridge et al., 2017) and tiger shark dives in West End, Grand Bahama (Hammerschlag et al., 2017). Despite rare-

species dives only generating ~18 % of the revenue of shark-dive tourism in The Bahamas, the importance of this income is greater in economically depauperate Family Islands where these interactions take place.

The ongoing capture of migratory shark species coupled with their economic value to the Bahamian economy highlights the need for the Bahamian government to engage further in regional collaborative management initiatives. The Bahamas has chaired the United Nations Save-Our-Sharks Coalition since 2013 and has advocated for the sustainable management and conservation of sharks at the United Nations via a series of meetings and workshops. The Bahamas is also a member of the CRFM and the Western Atlantic Fisheries Commission (WECAFC) but is not currently a member of the International Commission for the Conservation of Atlantic Tunas (ICCAT) that manages all high seas fisheries in the region. More recently, The Bahamas officially co-sponsored proposals resulting in the successful listing of the silky shark, *Carcharhinus falciformis* (Müller & Henle), common thresher shark, *Alopias vulpinus* (Bonnaterre) and devil rays (Mobulidae) in the CITES Appendices at CITES COP16. Given the importance of sharks and other highly migratory species such as tunas (Scombridae) and billfish (Istiophoridae and Xiphiidae; Genter, 2016) to the Bahamian economy, it is imperative that The Bahamas becomes an active participant in the regional management of these species to ensure that sustainable management and conservation practices are extended throughout their range.

3.2. *Sea turtle research and conservation*

Four species of sea turtles – green, *Chelonia mydas* (Linnaeus), loggerhead, *Caretta caretta* (Linnaeus), hawksbill, *Eretmochelys imbricata* (Linnaeus) and leatherback, *Dermochelys coriacea* (Vandelli) – occupy Bahamian waters (Lahanas et al., 1998, McClenachan et al., 2006; Dodge et al., 2014). Historically, turtles and their eggs were harvested as a source of food or income for local fishers (Campbell, 2002). Since 1986, it has been illegal to kill adult hawskbill turtles or harvest their eggs. While landings data for sea turtles in The Bahamas are not comprehensive, DMR reports a peak of 52 metric tonnes of sea turtles landed in 1985 declining to 1 metric tonne in 2008 (DMR unpubl. data). In response to severe declines in global populations, the

Government of The Bahamas passed legislation in 2009 providing full protection for all sea turtles found in Bahamian waters, making it illegal to harvest marine turtles or buy and sell any marine turtle products, but poaching of sea turtles and their eggs continues (Stephen Connett, Archie Carr Center for Sea Turtle Research, pers. comm.).

How the 2009 ban on sea turtle harvest has affected populations in The Bahamas is not entirely clear due to the prolonged life histories and transboundary movements typical of these species (Chaloupka et al., 2008). Emerging results indicate possible increases in subpopulations of juvenile green sea turtles since 2009, however, these data must be interpreted cautiously as long-lived species such as turtles take many years for populations to recover (Chaloupka et al., 2008) and sea turtle growth rates are showing significant decline in relation to increasing sea surface temperatures (Bjorndal et al., 2017). Immigration of new recruits is also dependent on nesting success in other countries throughout the Caribbean and United States (Lahanas et al., 1998) and in 2015 green turtle nesting broke records with 14,152 nests recorded in the Archie Carr National Wildlife Refuge, where approximately 35 % of all green sea turtles nest in Florida (https://conserveturtles.org/archie-carr-refuge-nesting-trends/). Long-term monitoring plans as well as multinational collaboration are essential for evaluating management efficacy (Blumenthal et al., 2006). Several long-term monitoring studies of sea turtle aggregations across the Bahamian archipelago are providing an understanding of demographic characteristics (e.g., immigration and emigration), which gives insight to the overall population status. For example, results of a long-term mark-recapture study in a protected area in the southern Bahamas found that changes in immigration, not survival or emigration, were responsible for a 38.8 % annual increase in the number of juvenile green sea turtles between 1979–1985. The population then decreased by 13.1 % annually until 1994 and numbers did not stabilize until 2001 (Bjorndal et al. 2005). This study determined that abundance can vary greatly despite long-term stability, so assessments over short time intervals can be misleading. Since the harvest ban has only been in place for 8 years, full effects of the ban may not be realised for 20 years or more, highlighting the necessity for long-term monitoring.

Sea turtles play broad ecological roles as consumers on seagrass pastures (Aragones et al., 2006) and sponges (León & Bjorndal, 2002), nutrient

enrichers of beach and dune systems during nesting (Vander Zanden et al., 2012) and as prey for various beach and marine predators. Research to date has focused on the foraging behaviour, movement patterns and growth rates of juvenile green and hawksbill sea turtles in tidal mangrove creeks and seagrass pastures (Bjorndal et al., 2000; Bjorndal & Bolten, 2010). Studies that estimate carrying capacities of different habitats, as well as the positive and negative effects (Lal et al., 2010; Heithaus et al., 2014) of sea turtles within marine ecosystems will aid in future conservation strategies of these species.

3.3. *Bonefish research and management*

Bonefish (*Albula* spp.) are the centrepiece of an economically and culturally important recreational flats fishery in The Bahamas (Fedler, 2010). Much of the historical fishing mortality on bonefish came from “hauling” (i.e., seining and block netting) and hand lining and catch was often consumed or sold to local communities (Danylchuk et al., 2008b). The development of the recreational fishery in the mid-1960s along with regulations enacted in 1986 that prohibited hauling and commercial sale of bonefish changed this fishery to primarily catch-and-release (BDF, 1986). Today, the vast majority of fishing pressure comes from recreational fishing with few subsistence fisherman that harvest bonefish for consumption (Danylchuk et al., 2008b). Overall, the fishery has evolved from primarily harvest to almost exclusively a high-value recreational catch-and-release sport fishery (Adams & Murchie, 2015).

This non-extractive fishery generates approximately US $140 million per year for The Bahamian economy, with most of the revenue going to Family Islands rather than the main population and tourism centres (Fedler, 2010). On some islands, these revenues are a substantial portion of overall tourism. For example, over 80 % of the tourism expenditures on Andros come from flats anglers that spend money on guides, food, accommodations, tackle and airfare (Fedler, 2010). The conservation status of bonefish remains unknown throughout much of the world, including The Bahamas. Recently, bonefish were listed as near threatened on the ICUN Red List of Threatened Species, with particular emphasis on declining bonefish stocks in the Florida Keys, St. Croix, Bermuda and the Yucatan Peninsula (Adams et al., 2012). Although the causes of declines are unclear, bonefish display a high degree of site fidelity, which

could make them particularly susceptible to habitat loss (Adams et al., 2012; Murchie et al., 2013). Bahamian bonefish stocks could face declines similar to those observed in Florida (see Santos et al., 2017) if conservation measures (e.g., habitat protection) are not implemented. Estimates of bonefish population structure and abundance in The Bahamas are priorities for determining their conservation status.

Substantial research on bonefish in The Bahamas has focused on best handling practices to ensure survival post-release, resulting in publication of a leaflet that has been shared with anglers, guides and lodges (Adams & Cooke, 2015). Key findings indicate that limiting air exposure, minimizing fight time, handling fish with wet hands, not using lip-gripping devices and fishing in locations with low predator densities improve survival (Cooke & Philipp, 2004; Cooke et al., 2008; Danylchuk et al., 2007; 2008a; Suski et al., 2007; Hannan et al., 2015). Based on anecdotal evidence (i.e., informal discussions with anglers and guides, blog photos and popular press articles), it appears that anglers and guides have adopted many of these best practices, however, future research should evaluate the extent of the best practices application. More recently, research priorities have shifted to focus on population connectivity by identifying migration corridors, spawning aggregations, larval dispersal and genetic structure throughout the region (Danylchuk et al., 2011; Murchie et al., 2013; 2015; Wallace & Tringali, 2016). Outcomes from these studies have the potential to influence the placement and management of MPAs that protect key habitats and stocks, thereby helping to conserve this species (Grüss et al., 2014). The Government of The Bahamas designated marine parks on the north and east side of Grand Bahama, southern Abaco, and the west side of Andros that will protect several bonefish migration routes and spawning aggregations.

Identifying additional foraging habitat, migration routes, spawning aggregations, and larval dispersal routes, particularly in the southern portion of the archipelago, should be the focus of future research. Lastly, research on the response of bonefish to climate change stressors in the nearshore environment has indicated that bonefish will likely be more vulnerable to increases in temperature than other fish species (Shultz et al., 2014; Shultz et al., 2016). Bonefish habitats that act as thermal refuges (e.g. deeper water and upwellings) may be critical to include in MPAs as sea surface temperature increases in the future. Overall, due to its economic and cultural importance, coupled with high

levels of catch and release by recreational anglers, this fishery has benefited from increased regulations by the DMR and self-regulation from the angling community (i.e., encouraging fellow anglers to follow best handling practices). Anglers and guides should unify and incorporate the best available science to lobby for improved regulations (e.g., fines for habitat destruction), additional enforcement and habitat protection to ensure that bonefish remain the centrepiece of Bahamian flats fisheries that benefit local economies.

4.0. Emerging fisheries

While traditional fishery taxa (e.g. Caribbean spiny lobster, groupers and snappers) are of greatest economic and cultural importance to the Bahamian fisheries sector, several new fisheries have recently emerged. These fisheries have become established due to declines in traditional fishery species and other influences including social and economic factors as well as advances in biomedical research, such as the use of bioactive compounds derived from marine organisms in drug development (e.g., Haefner, 2003). Emerging fisheries have the potential to expand the fishing sector, improve food security and provide income to a greater number of fishers. However, they present new challenges for management due to lack of data on landings, population dynamics and the ecological function of these species. Some examples of emerging fisheries include parrotfishes, sea cucumbers and gorgonians. Two examples of emerging fisheries within The Bahamas are presented.

4.1. *Parrotfishes*

As recently as the mid-2000s, parrotfishes (Scarinae) were only taken as bycatch in fish pots and occasionally used for bait (Mumby et al., 2006). Over the past decade, however, large parrotfish species, such as stoplight parrotfish, *Sparisoma viride* (Bonnaterre), are commonly found at local landing sites and fish markets for sale on several islands, and surgeonfishes (Acanthuridae) are also seen on occasion (Craig Dahlgren, Perry Institute for Marine Science, unpubl. data). The development of this fishery is of concern due to the ecological role that herbivores play as grazers on coral reefs. As high abundances of large parrotfishes are linked to decreases in macroalgal cover

and increases in coral recruitment (Mumby et al., 2006; Mumby et al., 2007), removal of individuals from the ecosystem may have detrimental effects. The role of parrotfishes as grazers is particularly important for reef health in The Bahamas, as other known important grazers such as the long-spined sea urchin, *Diadema antillarum* Philippi, are rare (Dahlgren *et al.*, 2016b). At present, The Bahamas has greater densities of large parrotfishes than other parts of the Caribbean (Dahlgren et al., 2016b), but the development of this emerging fishery poses a danger to these populations and the ecological function that they serve. Research is currently underway to assess the harvest of parrotfish, including how it varies across The Bahamas, which species are being targeted and how the development of the fishery is affecting populations.

While studies into the extent of this fishery and factors driving its emergence have only just begun, contributing issues are likely the depletion of other fishery resources and an increased demand for parrotfish among immigrants from Haiti and other parts of the Caribbean where parrotfishes are a traditional food (e.g., Ferry & Kohler, 1987; Hawkins & Roberts, 2004). Other countries around the region, including Belize, Bonaire, Bermuda and the Dominican Republic have either banned parrotfish fishing or imposed gear restrictions to limit their harvest (e.g., Jackson et al., 2014). Studies from Bermuda illustrate how fishing has reduced biomass and skewed sex ratios of parrotfish, though these effects may be reversible over 3–6 years following a fishing ban (O'Farrell et al., 2015a, b). While the parrotfish fishery currently serves an emerging domestic market, and may be developing as an export fishery, management decisions must examine its value as a commercial fishery weighed against its ecological value in maintaining the health of coral reefs (Bozec et al., 2016) and the ecosystem services that reefs provide to The Bahamas.

4.2. *Sea cucumbers*

New access to international markets by Bahamian fishers has led to a fishery for holothurians or sea cucumbers, which are a valuable commodity in many Asian markets. Unfortunately, due to density-dependent reproduction, many sea cucumbers stocks are easily overfished and have very slow rates of recovery (e.g., Friedman et al., 2011). Sea cucumbers play an important

ecological role in tropical marine systems as bioturbators and processors of detritus, thereby altering infaunal communities (Dahlgren et al., 1999) and enhancing benthic microalgae and eelgrass growth (Wolkenhauer et al., 2010). Loss of sea cucumbers may have significant ecological consequences for other species that live or feed in soft substrates or seagrass habitats.

In 2010, a small-scale export fishery for sea cucumbers opened in north Andros targeting two commercially valuable shallow water species, the donkey dung or "brown" sea cucumber, *Holothuria mexicana* Ludwig, and the furry or "green" sea cucumber, *Astichopus multifidus* (Sluiter). This fishery engaged at least 120 fishers using small boats (2.5–7.6 m) on day trips with prices that typically varied from $0.20 to $0.45 per sea cucumber (Craig Dahlgren, Perry Institute for Marine Science & Lester Gittens, DMR, unpubl. data). At the start of the fishery, non-conventional fishers, including women and children, gathered sea cucumbers by wading in shallow water while the traditional fishers, primarily men, gathered conch and other species. Subsequently, shallow areas were rapidly depleted and the fishery moved to water depths only accessible by free diving. Between February and July 2010, total sea cucumber landings were reduced by 60 % as fishers dropped out of the fishery and distance travelled to sustain high landings increased throughout the year (Dahlgren, 2010; Craig Dahlgren, Perry Institute for Marine Science & Lester Gittens, DMR, unpubl. data). After only 11 months, the fishery collapsed, due to local stock depletion, high fuel costs and falling sea cucumber prices. By November 2010, stocks had been depleted to the point where sea cucumber densities in fished areas were 77–83 % lower than unfished areas (Craig Dahlgren, Perry Institute for Marine Science & Lester Gittens, DMR, unpubl. data). Although this fishery was not successful, increased demand from Asia and favourable economic relations between The Bahamas and China have revived interest in sea cucumber harvesting. Since 2016, there have been reports of sea cucumbers being harvested in several parts of The Bahamas but no data on landings have been collected. Because sea cucumber fisheries around the world have proven difficult to manage sustainably, it may not be suitable for further development in The Bahamas unless better stock assessments and strict limits are placed on the fishery (Anderson et al., 2011; Purcell et al., 2013).

5.0. Management Recommendations

For both existing and emerging fishery species, there is a disparity between information required for effective species-specific management and the scale on which monitoring efforts and research are conducted in the country because of limited financial resources, reduced technical capacity, time and other logistical constraints. To better address fisheries objectives for The Bahamas (see Waugh et al. 2010) and prevent further declines in species and ecosystem function, sufficient data for more accurate stock assessments is needed to inform management strategies and harvest regulations for commercially important species. Integrative and inter-disciplinary monitoring and research approaches are recommended to address multiple questions and potentially alleviate costs. For example, population genetics coupled with traditional and emerging *in situ* monitoring technologies (e.g. hydroacoustics and telemetry) are likely to help with stock identification and long-term monitoring of population status for species of interest (Paris et al., 2018).

From a management perspective, critical issues common across all fisheries sectors relate to the government's capacity to enforce existing regulations and address IUU fishing. RBDF is currently in the process of completing base repairs to its headquarters in New Providence and establishing another post in the southern Bahamas to increase its surveillance and enforcement capabilities. These improvements, along with training programs for enforcement officers (including DMR fishery officers), should help address problems of IUU fishing (local and foreign) throughout the archipelago. New technologies, such as vessel monitoring systems and drones, along with additional water-based patrol capacities, are also important for monitoring and deterring illegal fishing activity. Regional (e.g., CRFM) and international (e.g., ICCAT) partnerships should be further explored to reduce the amount of IUU fishing and protect highly migratory species. However, more emphasis needs to be placed on routine data collection and management systems across DMR and enforcement agencies to better monitor and regulate fishing activity (e.g. through licensing of all fishing vessels, gears and recreational fishers) and to promote consistency, accuracy and timely reporting across all fishery sectors (e.g. via standardised reporting systems such as the newly implemented Fisheries Management and Information Systems (FISMIS) for DMR).

Countries with limited means for conventional fisheries management, including The Bahamas, are often particularly interested in the fisheries roles of MPAs. For example, in areas of The Bahamas lacking robust fisheries enforcement, MPAs are currently the main management tool being used to promote fisheries sustainability (e.g., Stoner et al. 2012c). Fisheries bioeconomic models suggest that for enhanced fisheries yields, MPAs should be placed where fish productivity and dispersal, via either larval or adult export, are high enough that fishers' foregone harvests can be compensated by consistently larger yields and profits from surrounding areas (e.g., Sanchirico & Wilen, 2001). Other analyses suggest that when economic benefit-sharing is structured in appropriate ways, ecotourism value can provide sustainable compensation for forgone fisheries extraction from no-take MPAs (Sala et al., 2016; Wabnitz et al., 2018). In addition, other social considerations, such as the design and implementation of locally appropriate MPA governance and management regimes are increasingly recognized as being important for MPA effectiveness through facilitation of public support and compliance (Bennett & Dearden, 2014; Kaplan et al. 2015).

Given biophysical and economic variability across MPAs systematic understanding of how such social factors influence MPA effectiveness for fisheries management and biological conservation objectives remains challenging and requires more careful and sophisticated approaches to design, monitoring and assessment of MPA management (Ahmadia et al. 2015). In the meantime, however, better integration of fisheries management and conservation goals in MPA planning is underway in The Bahamas (Green et al., 2016; Knowles et al., 2017), and further stakeholder engagement and strengthening of more integrated management offers the promise of simultaneously enhancing both MPA and fisheries management (Weigel et al., 2014; Brumbaugh 2017).

6.0. Conclusion

The future of fisheries depends on the successful use of adaptive measures to address both current and predicted anthropogenic and natural impacts to species and their habitats. In The Bahamas and other SIDS, exploited species provide key ecological functions that are critical to maintain

healthy marine ecosystems. Their continued overexploitation therefore, beyond reducing stock productivity and prolonging recovery, may also reduce ecosystem resilience. In contrast to most single- or even multi-species management approaches, ecosystem-based fisheries management attempts to integrate more ecosystem components so that unintended ecological impacts from fishing can be minimized, trends in ecosystems can be better predicted and other human interests and associated ecosystem services can be included and sustained. Precautionary and ecosystem-based approaches should be applied to all species and habitats, especially where data are limited or non-existent, to promote sustainable fisheries and maintain biodiversity. The use of MPAs is a good example of these approaches, but to be effective for managing fisheries, conserving biodiversity and protecting ecosystem function, MPAs need to be well designed, managed and prohibitive of activities that are extractive or degrade habitat quality.

Finally, in addition to the need for more targeted science and complementary pre-cautionary management policies, scientific reasoning needs to be more accessible to policymakers and the public. Scientists and environmental organizations must therefore craft and deliver a range of succinct, science-grounded messages targeting multiple audiences to support legislation for species and ecosystem sustainability. Such a multi-faceted approach will help ensure that culturally and economically important natural resources will be available for future generations. The Bahamas has made considerable progress towards assessing the status of some species and protecting key habitats. While additional research is required, preliminary results have highlighted both successful conservation actions and areas where fisheries regulations can be improved. Moving forward, the development and implementation of species-specific fishery regulations, national management plans (and where appropriate, regional plans), as well as greater exploration and development of ecosystem-based management approaches, will be important approaches to promote recovery and sustainability for current and emerging Bahamian fisheries and the ecosystems on which they depend.

Acknowledgements

We thank the Department of Marine Resources for providing fishery data. Figure 1 was kindly prepared by Lindy Knowles (Bahamas National Trust). Feedback from three reviewers and the editor helped improved the quality of this manuscript.

References

Adams, A., & Cooke, S. J. (2015). Advancing the science and management of flats fisheries for bonefish, tarpon, and permit. *Environmental Biology of Fishes* **98,** 2123–2131.

Adams, A., Guindon, K., Horodysky, A., MacDonald, T., McBride, R., Shenker, J., & Ward, R. (2012). *Albula vulpes*. The IUCN Red List of Threatened Species. Version 2015.1.

Adams, A. J., & Murchie, K. J. (2015). Recreation fisheries as conservation tools for mangrove habitats. *American Fisheries Society Symposium* **83**, 43–56.

Ahmadia, G.N., Glew, L., Provost, M., Gill, D., Hidayat, N.I., Mangubhai, S., Purwanto, & Fox, H.E. (2015). Integrating impact evaluation in the design and implementation of monitoring marine protected areas. *Philosophical Transactions of the Royal Society B: Biological Sciences* **370**(1681), 20140275. http://dx.doi.org/10.1098/rstb.2014.0275

Albins, M. A., & Hixon, M. A. (2013). Worst case scenario: potential long-term effects of invasive predatory lionfish (*Pterois volitans*) on Atlantic and Caribbean coral-reef communities. *Environmental Biology of Fishes* **96**, 1151–1157.

Anderson, S. C., Flemming, J. M., Watson, R., & Lotze, H. K. (2011). Serial exploitation of global sea cucumber fisheries. *Fish and Fisheries* **12**, 317–339.

Anon (Anonymous). (2008). Report of 4th Annual Scientific Meeting – Kingston, St. Vincent and the Grenadines, 10-20 June 2008. National Reports. CRFM Fishery Report 2008. Vol 1 Suppl.1, Belize City 76pp.

Anon (Anonymous). (2010). Department of Statistics All Bahamas Population and Housing Census Report 2010.

Anon. (Anonymous). (2012). Department of Statistics Occupations and Wages in the Accommodation and Food Activities Industry Report 2012.

Anon. (Anonymous). (2014). Department of Statistics National Accounts Environmental Review Report 2014.

Aragones, L. V., Lawler, I. R., Foley, W. J., Marsh, H. (2006). Dugong grazing and turtle cropping: grazing optimization in tropical seagrass systems? *Oecologia* **149**, 635–647.

BDF (Bahamas Department of Fisheries). (1986). Bahamian Fisheries Regulations and Reports. Nassau, Bahamas.

Bennett, N.J., & Dearden, P. (2014). From measuring outcomes to providing inputs: Governance, management, and local development for more effective marine protected areas. *Marine Policy* **50** (Part A), 96–110.

Bjorndal, K. A., & Bolten, A. B. (2010). Hawksbill sea turtles in seagrass pastures: success in a peripheral habitat. *Marine Biology* **157**, 135–145.

Bjorndal, K. A., Bolten, A. B., & Chaloupka M. Y. (2000). Green turtle somatic growth model: evidence for density dependence. *Ecological Applications* **10**, 269–282.

Bjorndal, K. A., Bolten, A. B., & Chaloupka, M. Y. (2005). Evaluating trends in abundance of immature green turtles, *Chelonia mydas*, in the Greater Caribbean. *Ecological Applications* **15**, 304–314.

Bjorndal, K.A., A.B. Bolten, M. Chaloupka, V.S. Saba, C. Bellini, M.A.G. Marcovaldi, A.J.B. Santos, L.F. Wurdig Bortolon, A.B. Meylan, P.A. Meylan, J. Gray, R. Hardy, B. Brost, M. Bresette, J.C. Gorham, S. Connett, B. Van Sciver

Crouchley, M. Dawson, D. Hayes, C.E. Diez, R.P. van Dam, S. Willis, M. Nava, K.M. Hart, M.S. Cherkiss, A.G. Crowder, C. Pollock, Z. Hillis-Starr, F.A. Muñoz Tenería, R. Herrera-Pavón, V. Labrada-Martagón, A. Lorences, A. Negrete-Philippe, M.M. Lamont, A.M. Foley, R. Bailey, R.R. Carthy, R. Scarpino, E. McMichael, J.A. Provancha, A. Brooks, A. Jardim, M. López-Mendilaharsu, D. González-Paredes, A. Estrades, A. Fallabrino, G. Martínez-Souza, G.M. Vélez-Rubio, R.H. Boulon, Jr., J.A. Collazo, R. Wershoven, V. Guzmán Hernández, T.B. Stringell, A. Sanghera, P.B. Richardson, A.C. Broderick, Q. Phillips, M. Calosso, J.A.B. Claydon, T.L. Metz, A.L. Gordon, A.M. Landry, Jr., D.J. Shaver, J. Blumenthal, L. Collyer, B.J. Godley, A. McGowan, M.J. Witt, C.L. Campbell, C.J. Lagueux, T.L. Bethel and L. Kenyon**.** (2017). Ecological regime shift drives declining growth rates of sea turtles throughout the West Atlantic. *Global Change Biology* **23**, 4556–4568.

Blumenthal, J. M., Solomon, J. L., Bell, C. D., Austin, T. J., Ebanks-Petrie, G., Coyne, M. S., Broderick, A. C., & Godley, B. J. (2006). Satellite tracking highlights the need for international cooperation in marine turtle management. *Endangered Species Research* **2**, 51–61.

Boudreau, S. A., & Worm, B. (2012). Ecological role of large benthic decapods in marine ecosystems: a review. *Marine Ecology Progress Series* **469**, 195–213.

Bozec, Y. M., O'Farrell, S., Bruggemann, J. H., Luckhurst, B.E., & Mumby, P. J. (2016). Tradeoffs between fisheries harvest and the resilience of coral reefs. *Proceedings of the National Academy of Sciences* **113,** 4536–4541.

Branch, T. A., Jensen, O. P., Ricard, D., Ye, Y., & Hilborn, R. A. Y. (2011). Contrasting Global Trends in Marine Fishery Status Obtained from Catches and from Stock Assessments. *Conservation Biology* **25**, 777–786.

Brumbaugh, D.R. (2017). Co-Management of Marine Protected Areas: A Suggested Framework for The Bahamas. Report to The Nature Conservancy,

Northern Caribbean Program, Nassau, Bahamas: 31 pp. http://dx.doi.org/10.13140/RG.2.2.26295.21928/1

Buchan, K. C. (2000). The Bahamas. *Marine Pollution Bulletin* **41**, 94–111.

Burgess, G. H., & Fordham, S. V. (2005). Regional overviews. In: Fowler, S.L., Cavanagh, R.D., Camhi, M., Burgess, G.H., Cailliet, G.M., Simpfendorfer, C.A., Musick, J.A. (Eds.), Sharks, Rays & Chimaeras: The Status of Chondrichthyan Fishes. IUCN/SSG Shark Specialist Group, Gland, Switzerland, 461 pp.

Butler, M., Cockcroft, A., MacDiarmid, A., & Wahle, R. (2011). *Panulirus argus*. The IUCN Red List of Threatened Species 2011: e. T169976A6697254. http://dx.doi.org/10.2305/IUCN.UK.2011-1.RLTS.T169976A6697254.en. Downloaded on 12 October 2016.

Callwood, K. A. (2010). Use of larval connectivity modelling to determine settlement habitats of *Panulirus argus* in The Bahamas as a pre-cursor to marine protect area network planning. Open access theses. Paper 59.

Callwood, K. A. (2016). Condos, connectivity, and catch: analyzing the state of the Bahamian spiny lobster fishery. Open access dissertations. Paper 1761.

Campbell, L. M. (2002). Contemporary culture, use, and conservation of sea turtles. In The Biology of Sea Turtles, Vol. II, 307-338 pp. CRC Press.

Carpenter, K. E., Claro, R., Cowan, J., Sedberry, G., & Zapp-Sluis, M. (2015). *Epinephelus striatus*. The IUCN Red List of Threatened Species 2015: e.T7862A70324790. Downloaded on 19 March 2018.

Chaloupka, M., Bjorndal, K. A., Balazs, G. H., Bolten, A. B., Ehrhart, L. M., Limpus, C. J., Suganuma, H., Troëng, S., & Yamaguchi, M. (2008). Encouraging outlook for recovery of a once severely exploited marine megaherbivore. *Global Ecology and Biogeography* **17**, 297–304.

Cheung, W. W. L., Sadovy, Y., Braynen, M. T., & Gittens, L. G. (2013). Are the last remaining Nassau grouper *Epinephelus striatus* fisheries sustainable? Status quo in the Bahamas. *Endangered Species Research* **20**, 27–39.

Cheung, W. W. L., Sarmiento, J. L., Dunne, J., Frölicher, T. L., Lam, V. W. Y., Deng Palomares, M. L., Watson, R., & Pauly, D. (2012). Shrinking of fishes exacerbates impacts of global ocean changes on marine ecosystems. *Nature Climate Change* **3**, 254−258.

Clark, S. A., Danylchuk, A. J., & Freeman, B. T. (2005). The harvest of juvenile queen conch (*Strombus gigas*) off Cape Eleuthera, The Bahamas: Implications for the effectiveness of a marine reserve. *Proceedings of the Gulf and Caribbean Fisheries Institute* **56**, 705−713.

Cooke, S. J., & Philipp, D. P. (2004). Behavior and mortality of caught-and-released bonefish (*Albula* spp.) in Bahamian waters with implications for a sustainable recreational fishery. *Biological Conservation* **118**, 599–607.

Cooke, S. J., Suski, C. D., Danylchuk, S. E., Danylchuk, A. J., Donaldson, M. R., Pullen, C., Bulté, G., O'Toole, A., Murchie, K. J., Koppelman, J. B., & Goldberg T.L. (2008). Effects of capture techniques on the physiological condition of bonefish (*Albula vulpes*) evaluated using field physiology diagnostic tools. Journal of Fish Biology **73**:1351−1375.

CRFM (Caribbean Regional Fisheries Mechanism). (2008). Report of Fourth Annual Scientific Meeting – Kingstown, St. Vincent and the Grenadines, 10-20 June 2008 – Fishery Management Advisory Summaries. CRFM Fishery Report – 2008, Vol. 2, 54 pp.

(CRFM) Caribbean Regional Fisheries Mechanism. (2014). Efforts implemented to prevent, deter and eliminate illegal, unreported and unregulated fishing in The Caribbean. CRFM Issues Paper No. 6, 8 pp.

Cushion, N. & Sullivan-Sealey, K. (2008). Landings, effort and socio-economics of a small scale commercial fishery in The Bahamas. *Proceedings of the Gulf and Caribbean Fisheries Institute* **60**, 162−166

Dahlgren, C. (2010). A study of the sea cucumber fishery of North Andros. Report to the Bahamas Department of Marine Resources. Nassau, Bahamas, 12 pp.

Dahlgren, C. P., Buch, K., Rechisky, E., & Hixon, M. A. (2016a). Multi-year tracking of Nassau grouper spawning migrations. *Marine and Coastal Fisheries* **8**, 522–535.

Dahlgren, C. P., Posey, M. H., & Hulbert, A. W. (1999). The effects of bioturbation on the infaunal community adjacent to an offshore hardbottom reef. *Bulletin of Marine Science* **64**, 21–34.

Dahlgren, C., Sherman, K., Lang, J., Kramer, P. R., & Marks, K. (2016b). Bahamas Coral Reef Report Card **1**, 2011–2013.

Danylchuk, A .J., Adams, A., Cooke., S. J., & Suski, C. D. (2008b). An evaluation of the injury and short-term survival of bonefish (*Albula* spp) as influenced by a mechanical lip-gripping device used by recreational anglers. *Fisheries Research* **93,** 248−252.

Danylchuk, A. J, Cooke, S. J., Goldberg, T. L., Suski, C. D., Murchie, K. J., Danylchuk, S. E., Shultz, A. D., Haak, C. R., Brooks, E. J., Oronti, A., Koppleman, J. B., & Philipp, D. P. (2011). Aggregations and offshore movements as indicators of spawning activity of bonefish (*Albula vulpes*) in The Bahamas. *Marine Biology* **158**, 1981–1999.

Danylchuk, A. J, Danylchuk, S. E., Cooke, S. J., Goldberg, T.L., Koppleman, J., & Philipp, D.P. (2008a). Ecology and management of bonefish (*Albula* spp) in the Bahamian Archipelago. In: Ault JS (ed) Ecology and Management of Bahamian Bonefish. CRC Press, Boca Raton, 79–92 pp.

Danylchuk, S. E., Danylchuk, A. J., Cooke, S. J., Goldberg, T. L., Koppelman, J., & Philipp, D. P. (2007). Effects of recreational angling on the post-release behavior and predation of bonefish (*Albula vulpes*): the role of equilibrium status at the time of release. *Journal of Experimental Marine Biology and Ecology* **346,** 127−133.

Dodge, K. L., Galuardi, B., Miller, T.J., & Lutcavage, M. E. (2014). Leatherback turtle movements, dive behavior, and habitat characteristics in ecoregions of the Northwest Atlantic Ocean. *PLoS ONE* **9**, e91726.

Douglas, C. H. (2006). Small island states and territories: sustainable development issues and strategies – challenges for changing islands in a changing world. *Sustainable Development* **14**, 75–80.

Ehrhardt, N. M. (2005). Population dynamic characteristics and sustainability mechanisms in key Western Central Atlantic spiny lobster, *Panulirus argus*, fisheries. *Bulletin of Marine Science* **76**, 501−526.

FAO (Food and Agriculture Organization). (1999). Fisheries and aquaculture issues in small island developing states. FAO Report COFI/99/7.

FAO (Food and Agriculture Organization). (2009). Fishery and aquaculture country profiles. Bahamas (2009). Country Profile Fact Sheets. In: FAO Fisheries & Aquaculture Department [online]. Rome. Updated 1 May 2009. [Cited 12 October 2016]. http://www.fao.org/fishery/facp/BHS/en#CountrySector-Statistics

FAO (Food and Agriculture Organization). (2016). Western Central Atlantic Fishery Commission. Sixteenth session of the commission report No. 1162. Guadeloupe, 20–24 June 2016.

FAO-FISHSTAT (Food and Agriculture Organization FISHSTAT). Global Capture Production (1950-2015). http://www.fao.org/fishery/statistics/global-capture-production/en)

Federal Register. (2016). Endangered and threatened wildlife and plants: final listing determination on the proposal to list the Nassau grouper as threatened under the Endangered Species Act. Federal Register **81**, 42268–42285.

Fedler, T. (2010). The economic impact of flats fishing in The Bahamas. Bahamas Flats Fishing Alliance, Nassau, Bahamas: 16 pp.

Ferry, R. E., & Kohler, C. C. (1987). Effects of trap fishing on fish populations inhabiting a fringing coral reef. *North American Journal of Fisheries Management* **7**, 580–588.

Friedman, K., Eriksson, H., Tardy, E., & Pakoa, K. (2011). Management of sea cucumber stocks: patterns of vulnerability and recovery of sea cucumber stocks impacted by fishing. *Fish and Fisheries* **12**, 75−93

Genter, B. (2016). The value of billfish resources to both commercial & recreational sectors in the Caribbean. FAO Fisheries & Aquaculture Circular No. 1125. Bridgetown, Barbados.

Green, A., Knowles, J., Dahlgren, C., Arnett, F., Knowles, L., & Albury-Smith, S. (2016). Bahamas protected: Realizing the 2020 goal to effectively manage and expand Bahamian marine protected areas. A report prepared for the Ministry of the Environment for The Bahamas on the Ecological Gap Analysis Workshop held in Nassau, New Providence (September 13-14th, 2016). The Nature Conservancy, Bahamas National Trust, and Bahamas Reef Environment Educational Foundation, Nassau, Bahamas: 41 pp.

Grüss, A., Robinson, J., Heppell, S., Heppell, S., & Semmens, B. (2014). Conservation and fisheries effects of spawning aggregation marine protected areas: What we know, where we should go, and what we need to get there. *ICES Journal of Marine Science* **71**, 1515–1534.

Guttridge, T. L., Van Zinnicq Bergmann, M. P. M., Bolte, C., Howey, L. A., Finger, J. S., Kessel, S. T., Brooks, J. L., Winram, W., Bond, M. E., Jordan, L. K. B., & Cashman, R.C. (2017). Philopatry and regional connectivity of the

Great hammerhead shark, *Sphyrna mokarran* in the US and Bahamas. *Frontiers in Marine Science* **4**, 10.3389/fmars.2017.00003.

Haas, A. R., Fedler, T., & Brooks, E. J. (2017). The contemporary economic value of elasmobranchs in The Bahamas: Reaping the rewards of 25 years of stewardship & conservation. *Biological Conservation* **207**, 55–63.

Haefner, B. (2003). Drugs from the deep: marine natural products as drug candidates. *Drug discovery today* **8**, 536–544.

Hammerschlag, N., Gutowsky, L. F. G., Gallagher, A. J., Matich, P., & Cooke, S. J. (2017). Diel habitat use patterns of a marine apex predator (tiger shark, *Galeocerdo cuvier*) at a high use area exposed to dive tourism. *Journal of Experimental Marine Biology and Ecology* **495**, 24–34.

Hannan, K. D., Zuckerman, Z. C., Haak, C. R., & Shultz, A. D. (2015). Impacts of sun protection on feeding behavior and mucus removal of bonefish, *Albula vulpes*. *Environmental Biology of Fishes* **98**, 2297–2304.

Hawkins, J. P., & Roberts, C. M. (2004). Effects of artisanal fishing on Caribbean coral reefs. *Conservation Biology* **18**, 215–226.

Heithaus, M. R., Alcoverro,T., Arthur, R., Burkholder, D. A., Coates, K. A., Christiansen, M. J., Kelkar, N., Manuel, S. A., Wirsing, A. J., Kenworthy, W. J., & Fourqurean, J. W. (2014). Seagrasses in the age of sea turtle conservation and shark overfishing. *Frontiers in Marine Science* **1**, 10.3389/fmars.2014.00028.

Hoegh-Guldberg, O., & Bruno, J. F. (2010). The Impact of Climate Change on the World's Marine Ecosystems. *Science* **5985**, 1523–1528.

Howey-Jordan, L. A., Brooks, E. J., Abercrombie, D. L., Jordan, L. K. B., Brooks, A., Williams, S., Gospodarczyk, E., & Chapman, D. D. (2013). Complex movements, philopatry and expected depth range of a severely threatened

pelagic shark, the Oceanic Whitetip (*Carcharhinus longimanus*) in the western north Atlantic. *PLoS One* **8**, e56588.

Jackson J. B. C., Donovan M. K., Cramer K. L., & Lam V.V. (eds). (2014). Status and Trends of Caribbean Coral Reefs:1970-2012. Global Coral Reef Monitoring Network, IUCN, Gland, Switzerland.

Kaplan, K.A., Ahmadia, G.N., Fox, H., Glew, L., Pomeranz, E.F., & Sullivan, P. (2015). Linking ecological condition to enforcement of marine protected area regulations in the greater Caribbean region. *Marine Policy* **62**, 186−195.

Knowles, J. E., Green, A. L., Dahlgren, C., Arnett, F., & Knowles, L. (2017). Expanding The Bahamas marine protected area network to protect 20 % of the marine and coastal environment by 2020: a gap analysis. 66 pp.

Kough, A. S., Cronin H., Skubel, R., Belak, C. A., & Stoner, A. W. (2017). Efficacy of an established marine protected area at sustaining a queen conch *Lobatus gigas* population during three decades of monitoring. *Marine Ecology Progress Series* **573**, 177–189.

Kough, A. S., Paris, C. B., & Butler, M. J. IV (2013). Larval connectivity and the international management of fisheries. *PLoS One* **8**, e64970. https:// 10.1371/journal.pone.0064970.

Lahanas, P. N., Bjorndal, K. A., Bolten, A. B., Encalada, S. E., Miyamoto, M. M., Valverde, R. A., & Bowen, B. W. (1998). Genetic composition of green turtle (*Chelonia mydas*) feeding ground population: Evidence for multiple origins. *Marine Biology* **130**, 345–352.

Lal, A., Arthur, R., Marbà, N., Lill, A. W. T, & Alcoverro, T. (2010). Implications of conserving an ecosystem modifier: Increasing green turtle (*Chelonia mydas*) densities substantially alters seagrass meadows. *Biological Conservation* **143**, 2730–2738.

Lapointe, B. E., Barile, P. J., Yentsch, C. S., Littler, M. M., Littler, D. S. & Kakuk, B. (2004). The relative importance of nutrient enrichment and herbivory on macroalgal communities near Norman's Pond Cay, Exumas Cays, Bahamas: a ''natural'' enrichment experiment. *Journal of Experimental Marine Biology and Ecology* **298***,* 275– 301.

Lea, J. S. E., Wetherbee, B. M., Queiroz, N., Burnie, N., Aming, C., Sousa, L. L., Mucientes, G. R., Humphries, N. E., Harvey, G. M., Sims, D. W., & Shivji, M., S. (2015). Repeated, long-distance migrations by a philopatric predator targeting highly contrasting ecosystems. *Nature Scientific Reports* **5**, 11202. https:// doi:10.1038/srep11202.

León, Y. M., & Bjorndal, K. A. (2002). Selective feeding in the Hawksbill turtle, an important predator in coral reef ecosystems. *Marine Ecology Progress Series* **245**, 249–258.

Lipcius, R. N., Stockhausen, W. T., & Eggleston, D.B. (2013). Metapopulation dynamics & marine reserves: Caribbean spiny lobster in Exuma Sound, Bahamas. In (Chapter 7 The Bahamas: Tropical Island Nation) Marine Conservation (Eds. Ray, G.C., & McCormick-Ray J.). Blackwell Scientific, Oxford, UK.

Mackey C., Dorsett, K., Lowe C., Saunders, K., Frith, I., Williams, L., & Newbold L. (2010). Census of population and housing. Department of Statistics. Government of The Bahamas. Nassau, Bahamas.

Marks, K.W., & Lang, J.C. (2016). AGRRA Summary Products, version (2016-08). Available online <http:www.agrra.org/data-explorer/explore-summary-products/ >

Maycock, V.D. (2016). Economic Impacts of Recreational Fishing in The Bahamas. Nassau: FAO and DMR. Published in FAO. 2016. Recreational fisheries economic impact assessment manual and its application in two study cases in the Caribbean: Martinique and The Bahamas, by Southwick, R., Maycock, D., & Bouaziz, M. FAO Fisheries and Aquaculture Circular No. 1128.

Bridgetown, Barbados.

McClenachan, L., Jackson, J. B. C., & Newman, M. J. T. H. (2006). Conservation implications of historic nesting beach loss. *Frontiers in Ecology and the Environment* **4**, 290–296.

Medley, P. A. H., & Gittens, L. (2012). Bahamas spiny lobster stock assessment. Department of Marine Resources, Nassau, Bahamas. July 2012.

Mintz, J. D., Lipcius, R. N., Eggleston, D. B., & Seebo, M. S. (1994). Survival of juvenile Caribbean spiny lobster: effects of shelter size, geographic location & conspecific abundance. *Marine Ecology Progress Series* **112**, 255–266.

MRAG Ltd. (2015). Revised action plan for Bahamas lobster fishery improvement project (FIP). Prepared for the WWF-US. Technical Report. London, UK. 37 pp.

Mumby, P. J., Dahlgren, C. P., Harborne, A. R., Kappel, C. V., Micheli, F., Brumbaugh, D. R., Holmes, K. E., Mendes, J. M., Broad, K., & Sanchirico, J. N. (2006). Fishing, trophic cascades, & the process of grazing on coral reefs. *Science* **311**, 98–101.

Mumby, P. J., Harborne, A. R., Williams, J., Kappel, C. V., Brumbaugh, D. R., Micheli, F. Holmes, K. E., Dahlgren, C. P., Paris, C. B., & Blackwell, P. G. (2007). Trophic cascade facilitates coral recruitment in a marine reserve. *Proceedings of the National Academy of Sciences* **104**, 8362–8367.

Murchie, K. J., Cooke, S. J., Danylchuk, A. J., Danylchuk, S. E., Goldberg, T. L., Suski, C. D., & Philipp, D. P. (2013). Movement patterns of bonefish (*Albula vulpes*) in tidal creeks and coastal waters of Eleuthera, The Bahamas. *Fisheries Research* **147**, 404–412.

Murchie, K. J., Shultz, A. D., Stein, J. A., Cooke, S. J., Lewis, J., Franklin, J., Vincent, G., Brooks, E. J., Claussen, J. E., & Philipp, D. P. (2015). Defining

adult bonefish (*Albula vulpes*) movement corridors around Grand Bahama in the Bahamian Archipelago. *Environmental Biology of Fishes* **98**, 2203 –2212.

Naro-Maciel, E., Reid, B., Holmes, K. E., Brumbaugh, D. R., Martin, M., & DeSalle, R. (2011). Mitochondrial DNA sequence variation in spiny lobsters: population expansion, panmixia, and divergence. *Marine Biology* **158***,* 2027–2041.

O'Farrell, S., Harborne, A. R., Bozec, Y. M., Luckhurst, B. E., & Mumby, P. J. (2015a). Protection of functionally important parrotfishes increases their biomass but fails to deliver enhanced recruitment. *Marine Ecology Progress Series* **522**, 245–254.

O'Farrell, S., Luckhurst, B. E., Box, S. J., & Mumby, P. J. (2015b). Parrotfish sex ratios recover rapidly in Bermuda following a fishing ban. *Coral Reefs* **35**, 421–425.

Paris, J. R., Sherman, K. D., Bell, E., Boulenger, C., Delord, C., El-Mahdi, M. B. M., Fairfield, E. A., Griffiths, A. M., Gutmann Roberts, C., Hedger, R. D., Holman, L. E., Hooper, L. H., Humphries, N. E., Katsiadaki, I., King, R. A., Lemopoulos, A., Payne, C. J., Peirson, G., Richter, K. K., Taylor, M. I., Trueman, C. N., Hayden, B., & Stevens, J. R. (2018). Understanding and managing fish populations: keeping the toolbox fit for purpose. *Journal of Fish Biology* **92**, 727–751.

Pauly, D., & Zeller, D. (2016). Catch reconstructions reveal that global marine fisheries catches are higher than reported and declining. *Nature Communications* **7**, 10244. https//doi:10.1038/ncomms10244.

Purcell, S. W., Mercier, A., Conand, C., Hamel, J.-F., Toral-Granda, M. V., Lovatelli, A., & Uthicke, S. (2013). Sea cucumber fisheries: global analysis of stocks, management measures and drivers of overfishing. *Fish and Fisheries* **14**, 34–59.

Sadovy, Y., & Colin, P. L. (1995). Sexual development and sexuality in the Nassau grouper. *Journal of Fish Biology* **46**, 961–976.

Sadovy, Y., & Eklund, A. M. (1999). Synopsis of biological data on the Nassau grouper, *Epinephelus striatus* (Bloch, 1792) and the Jewfish, *E. itajara*, Lichtenstein, 1822. 1–64 pp.

Sadovy de Mitcheson, Y., & Colin, P. L. (eds.) (2012). Reef Fish Spawning Aggregations: Biology, Research and Management. Vol. 35. Fish and Fisheries Series. Springer, New York 621 pp.

Sadovy de Mitcheson, Y., Craig, M. T., Bertoncini, A. A., Carpenter, K. E., Cheung, W. L. L., Choat, J. H., Cornish, A. S., Fennessy, S. T., Ferreira, B. P., Heemstra, P. C., Liu, M., Myers, R. F., Pollard, D. A., Rhodes, K. L., Rocha, L. A., Russell, B. C., Samoilys, M. A., & Sanciangco, J. (2013). Fishing groupers towards extinction: a global assessment of threats and extinction risks in a billion dollar fishery. *Fish and Fisheries* **14**, 119–136.

Sala, E., Ballesteros, E., & Starr, R. M. (2001). Rapid decline of Nassau Grouper spawning aggregations in Belize: fishery management and conservation needs. *Fisheries* **26**, 23–30.

Sala, E., Costello, C., De Bourbon Parme, J., Fiorese, M., Heal, G., Kelleher, K., Moffitt, R., Morgan, L., Plunkett, J., Rechberger, K.D., Rosenberg, A.A., & Sumaila, R. (2016). Fish banks: An economic model to scale marine conservation. *Marine Policy* **73**, 154–161.

Sanchirico, J. N., & Wilen, J. E. (2001). A bioeconomic model of marine reserve creation. *Journal of Environmental Economics and Management* **42**, 257–276.

Santos, R. O., Rehage, J. S., Adams, A. J., Black, B. D., Osborne, J., & Kroloff, E. K. N. (2017). Quantitative assessment of a data-limited recreational bonefish fishery using a time-series of fishing guides reports. *PLoS ONE* **12**(9): e0184776

Sherman, K. D., Dahlgren, C. P., & Knowles, L. C. (2018). Nassau grouper (*Epinephelus striatus*) conservation management plan for the Commonwealth of The Bahamas. Prepared for the Department of Marine Resources, Nassau, Bahamas.

Sherman, K. D., Dahlgren, C. P., Stevens, J. R., & Tyler, C. R. (2016). Integrating population biology into conservation management for endangered Nassau grouper *Epinephelus striatus*. *Marine Ecology Progress Series* **554**, 263–280.

Shipley, O. N., Howey, L. A., Tolentino, E. R., Jordan, L. K., Ruppert, J. L., & Brooks, E. J. (2017). Horizontal and vertical movements of Caribbean reef sharks (*Carcharhinus perezi*): conservation implications of limited migration in a marine sanctuary. *Royal Society Open Science* **4**, 160611.

Shultz, A. D., Zuckerman, Z. C., Stewart, H. A., & Suski, C. D. (2014). Seasonal blood chemistry response of sub-tropical nearshore fishes to climate change. *Conservation Physiology*, **2**, 1–12.

Shultz, A. D., Zuckerman, Z. C., & Suski, C. D. (2016). Thermal tolerance of nearshore fishes across seasons: implications for coastal fish communities in a changing climate. *Marine Biology*, **163**, 83.

Smith, N. S., & Zeller, D. (2013). Bahamas catch reconstruction: fisheries trends in a tourism-driven economy (1950-2010). Fisheries Centre, University of British Columbia. Working Paper #2013-08.

Stallings, C. D. (2009). Fishery-independent data reveal negative effect of human population density on Caribbean predatory fish communities. *PLoS One* **4**, e5333.

Stoner, A., Davis, M., & Booker, C. J. (2009). Queen Conch Stock Assessment: Proposed MPA and Fishing Grounds Berry Islands, Bahamas. Prepared by Community Conch. Submitted to the Government of The Bahamas. Retrieved

Oct 1, 2012 from http://www.community conch.org/wp-content/uploads/2010/10/AndrosReport.pdf.

Stoner, A., Davis, M. H., & Booker, C. J. (2012b). Negative consequences of allee effect are compounded by fishing pressure: comparison of queen conch reproduction in fishing grounds and a marine protected area. *Bulletin of Marine Science* **88**, 89–104.

Stoner, A., Davis, M. H., & Booker, C. J. (2012c). Abundance and population of queen conch inside and outside a marine protected area: Repeat surveys show significant declines. *Marine Ecology Progress Series* **460**, 101–114.

Stoner, A. W., Davis, M. H., & Booker, C. J. (2013). Queen conch stock assessment: Jumentos Cays and Ragged Islands, Fishing grounds in The Bahamas, June 2013. Prepared by Community Conch. Submitted to Bahamas Department of Marine Resources and Bahamas National Trust. Retrieved Feb. 28, 2017 from: http://www.communityconch.org/wp-content/uploads/2014/01/Jumentos-Ragged-Island-Report_FINAL-web.pdf. Nassau, Bahamas 30pp.

Stoner, A. W., Davis, M. H., & Booker, C. J. (2015). Queen conch stock assessment: Eastern Sand Bores, Tongue of the Ocean, Bahamas. Report for the Bahamas Department of Marine Resources. Retrieved on Dec. 3, 2017 from: http://www.communityconch.org/wp-content/uploads/2016/01/Community-Conch-Eastern-Sand-Bores-Report-121015.pdf. Nassau, Bahamas 16pp.

Stoner, A., Mueller, K., Brown-Peterson, N., Davis, M., & Booker. C. J. (2012a). Maturation and age in queen conch (*Strombus gigas*): urgent need for changes in harvest criteria. *Fisheries Research* **131–133**, 76–84.

Stump, K., Dahlgren, C. P., Sherman, K. D., & Knapp, C. (2017). Nassau grouper migrations during full moon suggest collapsed historic fish spawning aggregation and evidence of undocumented aggregation. *Bulletin of Marine Science* **93**,375–389.

Sullivan, M. (2013). Seafood Watch Seafood Report: Caribbean spiny lobster *Panulirus argus* Bahamas, Belize, Brazil, Honduras, and Nicaragua. Monterey, CA: Seafood Watch, Monterey Bay Aquarium.

Suski, C. D., Cooke, S. J., Danylchuk, A. J., O'Connor, C. M., Gravel, M. A., Redpath, T., Hanson, K. C., Gingerich, A. J., Murchie, K. J., Danylchuk, S. E., & Koppelman, J. B. (2007). Physiological disturbance & recovery dynamics of bonefish (*Albula vulpes*), a tropical marine fish, in response to variable exercise and exposure to air. *Comparative Biochemistry Physiology Part A: Molecular adn Integrative Physiology* **148**, 664–673.

Theile, S. (2005). Status of queen conch (*Strombus gigas*) stocks, management and trade in the Caribbean: A CITES review. *Proceedings of the Gulf and Caribbean Fisheries Institute* **56**, 675–694.

Thomas, C. L., Auscavitch, S., Brooks, A., & Stoner, A. (2015). Assessing essential queen conch (*Strombus gigas*) habitat in Eleuthera, The Bahamas: population declines suggest the urgent need for changes in management. *Proceedings of the Gulf and Caribbean Fisheries Institute* **67**, 344–347.

Vander Zanden, H. B., Bjorndal, K. A., Inglett, P. W., & Bolten, A. B. (2012). Marine-derived Nutrients from Green Turtle Nests Subsidize Terrestrial Beach Ecosystems. *Biotropica* **44**, 294–301.

Wabnitz, C.C.C., Cisneros-Montemayor, A.M., Hanich, Q., & Ota, Y. (2018). Ecotourism, climate change and reef fish consumption in Palau: Benefits, trade-offs and adaptation strategies. *Marine Policy* **88**, 323–332.

Wallace, E. M., & Tringali, M. D. (2016). Fishery composition and evidence of population structure and hybridization in the Atlantic bonefish species complex (*Albula* spp.)*. Marine Biology* **163**, 142. https://DOI 10.1007/s00227-016-2915-x.

Waugh, G.T., Braynen, M.T., Bethel, W.G., & Gittens, L. (2010). The Government of The Bahamas, The Ministry of Agriculture and Marine

Resources: Department of Marine Resources Final five year sector strategic plan 2010-2014. Nassau, Bahamas.

Weigel, J-Y, Mannle, K.O., Bennett, N.J., Carter, E., Westlund, L., Burgener, V., Hoffman, Z., Simão Da Silva, A., Kane, E.A., Sanders, J., Piante, C., Wagiman, S., & Hellman, A. (2014). Marine protected areas and fisheries: Bridging the divide. *Aquatic Conservation: Marine and Freshwater Ecosystems* **24**(S2), 199−215. http://dx.doi.org/10.1002/aqc.2514

Wolkenhauer, S-M., Uthicke, S., Burridge, C., Skewes, T., & Pitcher, R. (2010). The ecological role of *Holothuria scabra* (Echinodermata: Holothuroidea) within subtropical seagrass beds. *Journal of the Marine Biological Association of the United Kingdom* **90**, 215−223.

Worm, B., Hilborn, R., Baum, J. K., Branch, T. A., Collie, J. S., Costello, C., Fogarty, M. J., Fulton, E. A., Hutchings, J. A., Jennings, S., Jensen, O. P., Lotze, H. K., Mace, P. M., McClanahan, T. R., Minto, C., Palumbi, S. R., Parma, A. M., Ricard, D., Rosenberg, A. A., Watson, R., & Zeller, D. (2009). Rebuilding global fisheries. *Science* **325**, 578–585.

WWF (World Wildlife Fund). (2013). FIP handbook – guidelines for developing fishery improvement projects. Washington, DC. 86 pp.

Supplementary Materials

Contemporary and emerging fisheries in The Bahamas – conservation and management challenges, achievements and future directions

List of Tables

Supplementary Table 1.

Taxon	Common name	Total weight (tonnes)	Annual mean weight (tonnes)	Total Value (USD)
Panulirus argus (Latreille)	Caribbean spiny lobster	57299	1273	1,354,252,329
Lobatus gigas (Linnaeus)	queen conch	11945	629	76,318,659
Lutjanidae	snappers	11933	628	50,880,424
Epinephelus striatus (Bloch)	Nassau grouper	5179	247	32,527,853
Menippe mercenaria (Say)	stone crab	944	50	18,021,152
Epinephelidae	groupers	2054	54	13,482,464
Unknown fish spp.		2143	113	6,011,490
Carangidae	jacks	1396	73	4,523,579
Haemulidae	grunts	1208	64	2,914,984
Lachnolaimus maximus (Walbaum)	hogfish	284	47	1,508,629
Balistes vetula Linnaeus	queen triggerfish	118	20	373,589
Sphyraena barracuda (Edwards)	great barracuda	43	7	118,741
Chelonia mydas (Linnaeus)	green turtle	20	2	118,633
Caretta caretta (Linnaeus)	loggerhead turtle	17	1	86,437
Chondrichthyes	sharks	13	1	62,065

Supplementary Table 2.

Taxon	Common name	IUCN designation	Closed season	Harvest restriction(s)	Prohibited gears
Chondrichthyes					
Carcharhinus longimanus (Poey)	oceanic whitetip shark	vulnerable	year-round		all
Carcharhinus perezi (Poey)	reef shark	near threatened	year-round		all
Galeocerdo cuvier (Péron & Lesueur)	tiger shark	near threatened	year-round		all
Sphyrna mokarran (Rüppell)	great hammerhead	endangered	year-round		all
Actinopterygii					
Acanthocybium solandri (Cuvier)	wahoo	least concern	none	foreign recreational fishing vessels limited to 6 fish per person, in any combination	SCUBA, spear-guns
Albula vulpes (Linnaeus)	bonefish	near threatened	none	illegal to buy or sell bonefish	SCUBA, spear-guns, nets

Carangidae	jacks		none	foreign recreational fishing vessels limited to 4.5 kg scalefish	SCUBA, spear-guns
Coryphaena hippurus Linnaeus	dolphin fish	least concern	none	foreign recreational fishing vessels limited to 6 fish per person, in any combination	SCUBA, spear-guns
Epinephelus striatus (Bloch)	Nassau grouper	critically endangered	December 1 - February 28	≥1.36 kg	SCUBA, spear-guns
Epinephelidae	groupers (excluding Nassau and black grouper)		none	≥1.36 kg; foreign recreational fishing vessels limited to 4.5 kg scalefish	SCUBA, spear-guns
Lutjanidae	snappers		none	foreign recreational fishing vessels limited to 4.5 kg scalefish	SCUBA, spear-guns
Megalops atlanticus Valenciennes	tarpon	vulnerable	none	foreign recreational fishing vessels limited to 4.5 kg scalefish	SCUBA, spear-guns

Mycteroperca bonaci (Poey)	black grouper	near threatened	none	≥1.4 kg; foreign recreational fishing vessels limited to 4.5 kg scalefish	SCUBA, spear-guns
Scarinae	parrotfish		none	none	SCUBA, spear-guns
Trachinotus falcatus (Linnaeus)	permit	least concern	none	foreign recreational fishing vessels limited to 4.5 kg scalefish	SCUBA, spear-guns
Reptilia					
Caretta caretta (Linnaeus)	loggerhead turtle	vulnerable	year-round		all
Chelonia mydas (Linnaeus)	green sea turtle	endangered	year-round		all
Dermochelys coriacea (Vandelli)	leatherback turtle	vulnerable	year-round		all
Eretmochelys imbricata (Linnaeus)	hawksbill turtle	critically endangered	year-round		all
Gastropoda					

Lobatus gigas (Linnaeus)	queen conch	not assessed	none	flared lip/ export quota regulated by the Department of Marine Resources and Caribbean Regional Fisheries Mechanism	SCUBA, spear-guns
Malacostraca					
Menippe mercenaria (Say)	stone crab	not assessed	June 1 - October 15	minimum harvestable claw is 102 mm; harvesting of females is prohibited	SCUBA, spear-guns
Panulirus argus (Latreille)	Caribbean spiny lobster	data deficient	April 1 - July 31	82.55 mm carapace length or 140 mm tail length; capture of egg bearing females is prohibited; export quota of 2,268 metric tonnes of lobsters tails (or its equivalent in whole or live weight) per season will take effect beginning	SCUBA, spear-guns

				(Aug 2018-Mar 2019)	
Holothuroidea					
Astichopus multifidus (Sluiter)	green sea cucumber	least concern	none	none	SCUBA, spear-guns
Holothuria mexicana Ludwig	donkey dung or brown sea cucumber	least concern	none	none	SCUBA, spear-guns
Anthozoa					
Antillogorgia elisabethae Bayer	gorgonian or sea plume	not assessed	none	none	SCUBA, spear-guns
Demospongiae					
Hippiospongia lachne Laubenfels	wool sponge	not assessed	none	none	SCUBA, spear-guns
Spongia barbara Duchassaing & Michelotti	hardhead sponge	not assessed	none	none	SCUBA, spear-guns
Spongia graminea Hyatt	grass sponge	not assessed	None	none	SCUBA, spear-guns

Chapter VII: General Discussion

Species conservation is a multifaceted process, requiring robust scientific data to help guide and evaluate management strategies that consider both biological and socioeconomic factors. The overall aim of this thesis was to use complementary approaches to generate information required to further advance management for critically endangered Nassau grouper (*Epinephelus striatus*) populations. The thesis work was focused on The Bahamas, as it represents an important area for the species. To this end, a suite of methods including population genetics, acoustic telemetry, spawning aggregation surveys, and stakeholder assessments were employed to evaluate the status of Nassau grouper. Here, a critical synthesis of these studies is presented along with an analysis of their limitations. The implications of the thesis findings for Nassau grouper management and future research needs are also discussed.

Identifying knowledge gaps

A thorough review of the reproductive and population biology of Nassau grouper was undertaken, which identified key areas that need to be addressed for advancing the state of knowledge for the species, namely — performing adequate monitoring and stock assessments, and understanding patterns that drive variability in spawning seasonality and migration, genetic diversity, and connectivity (Chapter I: Sherman et al. 2016). This culminated in suggested recommendations for addressing issues/challenges that constrain progress for Nassau grouper conservation, which also have applications for other fishery species.

*Genetic population dynamics of Nassau groupe*r

How genetically diverse and connected are Nassau grouper populations within The Bahamas? To answer this question, putatively neutral polymorphic microsatellite markers were used to describe genetic diversity and differentiation throughout The Bahamas and to establish the first published estimates of effective population size (N_e) for Nassau grouper. Key findings from this research revealed that Bahamian Nassau grouper are relatively diverse, but exhibit low allelic

richness and show some signs of inbreeding depression (Chapter II: Sherman et al. 2017). Moreover, microsatellite data suggest that contemporary connectivity was probably higher than previously thought, and Nassau grouper have experienced substantial reductions in N_e as well as contemporary and historic bottlenecks (Sherman et al. 2017). We reconstructed historic estimates of N_e for Nassau grouper based on *VarEff* modelling and used this as a context for evaluating contemporary patterns of N_e, representing important new contributions to population genetics for the species. Frankham (2014) argued that N_e estimates greater than or equal to 1,000 should be sufficient for the maintenance of genetically viable populations in the long-term, but analysis of temporal changes in N_e for Nassau grouper highlighted the critical importance of understanding evolutionary history. Reduced allelic richness along with small contemporary estimates of N_e (compared to historic levels) suggests that the adaptive capacity of Nassau grouper to successfully navigate future environmental disturbances and/or increased stressors may be compromised (Sherman et al. 2017).

Building on the microsatellite work, this thesis then provided a genome-wide assessment of diversity and differentiation via restriction-site-associated DNA sequencing (RAD-seq), and characterised population structure, produced N_e estimates and performed the first analysis of contemporary gene flow for Nassau grouper populations in The Bahamas (Chapter IV). Utilising more than 13,000 SNPs, we found genomic estimates of diversity to be congruent with microsatellite results presented in Chapter II (Sherman et al. 2017). Moreover, SNP data revealed patterns of genetic population structure that were not apparent from microsatellite analysis and corroborated higher levels of genetic connectivity. Collectively, these results allude to within-country population sub-structuring that may be linked to adaptive selection, although the exact mechanisms driving this pattern are unclear and warrant further research.

FSA demographics, spawning stock sizes and migration patterns

Acoustic telemetry and diver surveys were used to evaluate the status of a historically important FSA – High Cay and describe movement patterns of Nassau grouper along one of the longest reef systems in The Bahamas (Chapter III: Stump

et al. 2017). Approximately 62 % of acoustically tagged Nassau grouper made multiple migrations along the continental shelf edge during the winter 2014-2105 spawning season covering an average of ~165 km (Stump et al. 2017). More importantly, telemetry data and underwater visual observations support the likely demise of this formerly active FSA, with movement patterns of Nassau grouper suggesting that another potential FSA may exist off the northern part of Andros (Stump et al. 2017).

Acoustic telemetry was also used to better understand migratory behaviour to and from an active Nassau grouper FSA located in the central Bahamas (Chapter IV). Using this technique, we were able to identify the origins of some aggregators and describe migratory patterns over the course of two spawning seasons. Findings from telemetry data imply that the Tongue of the Ocean and Exuma Sound are important migratory corridors for Nassau grouper during the spawning season. Whether this pattern holds true for other Nassau grouper FSAs within the country warrants investigation, but the emerging data suggest that conservative management strategies be applied to prevent habitat degradation and provide better protection for spawning routes and FSAs.

Addressing potential barriers to marine resource management

Humans are intrinsically linked to the environment and, as stakeholders, have the potential to hinder or support management policies aimed at protecting ecosystems and the species that reside within them (Hayes et al. 2005; Hilborn et al. 2005; Turner et al. 2014; Selkoe et al. 2016). Thus, understanding the motivations and perspectives of stakeholders is of critical importance. To this end, stakeholder assessments on the status and management of Nassau grouper in The Bahamas were performed to explore similarities and differences across individuals tasked with managing the fishery and imparting information to the general public (Chapter V). This research highlighted variability across conservation and enforcement stakeholders with regards to the perceived status of Nassau grouper and how the Bahamian fishery should be managed. Perceived threats to the species (e.g., FSA fishing, invasive alien species (IAS), and inadequate enforcement) were similar across stakeholders, reflecting a shared

understanding of these issues. Considerable support for science-based changes to current Bahamian fishery regulations along with the implementation of new regulations, and increased capacity to better enforce and manage the fishery were also mutually agreed upon strategies.

Finally, my thesis work examined fisheries governance in The Bahamas and highlighted examples of both successful and unsuccessful strategies that have been used to manage a range of fishery resources within the country (Chapter VI: Sherman et al. In Review). Synthesis of available commercial landings data along with a review of relevant peer-reviewed and grey literature were used to show how poorly defined fishery regulations, and weak management and enforcement frameworks have had limited success for species conservation. In contrast, proactive management strategies that are adequately communicated to stakeholders and better enforced have had better success. This synopsis led to recommendations that could be enacted to improve fisheries governance in The Bahamas including the application of inter-disciplinary approaches and increased cross-sectoral support for enforcement, education, outreach and advocacy.

Limitations and Directions for Future Research

Sampling design

For the research presented here, low sample sizes (i.e. <20) for several islands (e.g., New Providence, Ragged Island) and limited spatial coverage across certain islands (e.g. Abaco, Grand Bahama and Long Island) could have potentially influenced analyses of the spatial differentiation of Nassau grouper. Future Nassau grouper sampling should aim to increase geographic coverage and sample sizes across a range of life histories from these islands, but also expand coverage to islands that have not been sampled (e.g., Mayaguana, Cat Island, Acklins and Crooked Island). Additionally, samples should be obtained from other active FSAs, as this would permit more robust assessments of genetic differentiation, diversity, gene flow and N_e through the ontogeny of the species.

Genetic population dynamics

Inferences of genetic population structure have been notoriously difficult for marine species, which typically exhibit both adult and larval dispersal, thus contributing to high levels of gene flow that can act against amassing differentiation at neutral loci (Allendorf et al. 2010; Hemmer-Hansen et al. 2014). In some instances, this has been overcome through the use of large sample sizes, increased numbers of molecular markers, and/or marker diversification (Haasl and Payseur 2011; Putman and Carbone 2014; Mascolino et al. 2016; Drinan et al. 2016). Mascolino et al. (2016) utilised microsatellite and mtDNA sequencing for population genetic analyses and to assess relatedness and parentage in Mediterranean damselfish (*Chromis chromis*). Their findings showed differences in reproductive strategies used by males, which contributed to reproductive success (Mascolino et al. 2016). In another study, Drinan et al. (2016) ascertained fine-scale genetic population structure for Pacific halibut (*Hippoglossus stenolepis*) by comparing results from both sequence-tag linked and anonymous microsatellites. They found significant differentiation between the Aleutian Islands compared to other parts of the species' range that appear to be associated with selection and were able to identify existing population structure within an established management unit (MU) (Drinan et al. 2016).

Since the advent of high-throughput sequencing, large SNP datasets have been generated for many species, and the resulting data have been used to expose genetic structuring, and to guide the development of conservation management strategies (e.g., McMahon et al. 2014; Flanagan et al. 2017). The use of SNPs for genetic stock discrimination has been more successful when putatively adaptive loci are used (Delmore et al. 2015; Moore et al. 2017), but there are circumstances where this may not be appropriate (reviewed by Flanagan et al. 2017). Recently, Delmore et al. (2015) applied SNPs to examine how differences in migratory behaviour influenced population structure and fitness in two sub-species of Swainson's thrushes. The authors found evidence of divergent selection, linking patterns of seasonal migration to genes (e.g. clock genes) associated with migratory traits (Delmore et al. 2015).

For species where genomic resources (e.g. a draft/full genome or transcriptome) exist, regions of the genome under selection and gene function can be better defined (Nielsen et al. 2009), as demonstrated by more Delmore et al. (2015). The development of the Atlantic salmon genome (*Salmo salar*) for example, has enabled the detection of adaptive loci that helped to clarify patterns of genetic diversity and regional population structure (Bourret et al. 2013). While no such work has been undertaken for Nassau grouper, research has been underway since 2011 to develop and annotate the genome for orange-spotted grouper (*E. coides*), a commercially valuable epinephelid used in aquaculture. Indeed, genetic resources are more advanced for orange-spotted grouper, and studies have used molecular approaches, including quantitative trait loci (QTL) mapping and double digest restriction-site-associated sequencing (ddRAD-seq), to create linkage maps and to identify 17 genes linked to growth (Yu et al. 2016). Undoubtedly, development and annotation of a genome for Nassau grouper would allow for rigorous exploration of genes associated with reproduction, physiology and other important biological functions, as well as correlations between fitness traits and adaptive variation. Until then, the orange-spotted grouper draft genome can be used as a proxy for Nassau grouper to potentially increase the resolution and applicability of data generated through RAD-seq analysis.

While temporal variability in N_e and tests for bottlenecks were performed with microsatellite data providing basic insights into the demographics of Nassau grouper (Chapter II: Sherman et al. 2017), time constraints did not permit thorough investigations of other aspects (e.g., migration, expansion and divergence times) of their demographic history using the richer SNP dataset. As an example, Excoffier et al. (2013) used fastsimcoal2 to reconstruct the demographic history of human populations in Africa using previously studied SNP panels. The approach supported divergences between the Yoruba and San populations that would have occurred approximately 110 years (Excoffier et al. 2013). Such data are useful for providing context that may enrich understanding of contemporary genetic patterns.

Prior to the research presented in this thesis, limited SNP data existed for Nassau grouper (Jackson et al. 2014) and to my knowledge no attempts had been made to incorporate population genetics into management planning for the

species. The current research (Chapter IV) expands upon the previous SNP work in several ways. Firstly, it notably improved geographic representation for one of the most important areas globally for the species, corroborated patterns of genetic diversity, and illuminated patterns of gene flow throughout The Bahamas. Secondly, the large SNP dataset generated by RAD-seq was able to reveal intraspecific population structure that was undetected by microsatellite analysis (Chapter II: Sherman et al. 2017). Finally, genomic data enabled detections of loci under balancing selection as well as loci potentially under divergent selection, which may be contributing to the observed patterns of population structure within the Bahamian archipelago.

However, other factors have also been shown to influence intraspecific population structuring such as bottlenecks and genome changes (Frazer and Rusello 2013). Therefore, further research is required to reveal the processes that may be driving selection in Nassau grouper. Several approaches have been recommended for non-model species such as seascape genetics, candidate gene analysis and RNA-seq. These techniques have the potential to better characterise the attributes of putative adaptive loci, and provide stronger evidence for functional links to adaptive traits (reviewed by Nielsen et al. 2009). Progress in this direction will help to determine whether the fine-scale genetic population structure of Nassau grouper is driven by adaptation to environmental conditions (linked to seasonal migratory behaviour), to sexual selection (linked to sexual competition during annual spawning events) or to a combination of both.

Genetic assignment testing, when successful, can be used to identify the origins of species of interest (e.g., Larson et al. 2014; Bekkevold et al. 2015; Benestan et al. 2015). Commonly used forms of individual and population assignment tests include parentage (e.g. Harrison et al. 2012) and mixed stock analyses (e.g. Bradbury et al. 2016), which have useful applications for the management of endangered species. More recently, advances in software for genetic analyses (Paris et al. 2018) and random forest machine learning (ML) algorithms coupled with genetic studies (e.g., Noia et al. 2017; Sylvester et al. 2017; Schrider and Kern 2018) have also emerged as promising tools for unravelling intraspecific patterns of differentiation and improving the accuracy of

assignment tests. Sylvester et al. (2017) used RAD-seq and random forest algorithms to firstly identify reduced panels of informative SNPs for two salmonid species –Atlantic salmon (*S. salar*) and chinook salmon (*Oncorhynchus tshawytscha*)– and then to perform assignment testing. Using this approach, (Sylvester et al. 2017) achieved a higher level (≥90 %) of population assignment for both species than traditional Global and pairwise F_{ST} ranking methods. Noia et al. (2017) also employed this strategy to discover 98 informative SNPs (12 of which were under selection) and used them to successfully differentiate between proximate caligid sea lice (*Lepeophtheirus salmonis*) populations that had previously shown weak population structuring. The successfulness of these studies is encouraging and offers an alternative for refining genomic regions under selection, which can be used for future assignment testing of Nassau grouper. Such approaches are computationally demanding (Sylvester et al. 2017), however, and would probably be best suited to collaborations between ecologists and bioinformaticians for efficiency and cost effectiveness.

Now, more substantive SNP data are available which can contribute to a growing genomic database to facilitate future evaluations of spatial and temporal population structure of Nassau grouper. This may be achieved through regional and international collaborations, applying molecular and ML techniques to assist with monitoring population dynamics of Nassau grouper throughout the species' range (e.g. FishPopTrace, http://fishpoptrace.jrc.ec.europa.eu/).

FSA monitoring and research

Aside from the normal logistical challenges that occur during fieldwork, poor weather conditions often impede *in situ* monitoring of FSAs during the entire reproductive season (November-March). The inability to consistently monitor Nassau grouper FSAs, through traditional field surveys, however, can be compensated through use of technology (e.g. acoustic telemetry or hydroacoustic surveys) if funding allows (Starr et al. 2007; Baran et al. 2017; Egerton et al. 2017; Paris et al. 2018). Telemetry arrays have the capacity to continuously track movement patterns of tagged fish and hydroacoustic surveys have the ability to rapidly assess fish distribution and abundance. When both methods are combined,

however, they often yield new insights into migration patterns such as timing and site fidelity and can also be used to verify diver observations (Taylor et al. 2006; Egerton et al. 2017). Therefore, increased partnerships with local and regional researchers are encouraged to develop arrays in key areas for investigations of migration patterns of Nassau grouper and other aggregating species that may be using the same FSAs.

For well-funded Nassau grouper projects in parts of the Caribbean, other techniques (e.g. hydroacoustics and laser point counts for size estimates) have also been paired with traditional FSA surveys to monitor the status of recovering Nassau grouper FSAs, thus providing another measure of fish biomass, which is likely to be less biased than diver interpretations of fish abundance and size (Taylor et al. 2006; Heppell et al. 2012; Egerton et al. 2017). However, use of any of these methods does not negate the need for traditional FSA monitoring techniques as research has shown that ground-truthing is still necessary to improve confidence in hydroacoustic data for spawning stock estimates (e.g. Ehrhardt and Deleveaux 2007).

In recent years, environmental DNA (eDNA) has been used as both a presence/absence marker and a proxy for fish abundance and distribution (Bylemans et al. 2016, Lacoursière-Roussel et al. 2016; Sakata et al. 2017). With genetic material available for Nassau grouper, it would be worthwhile to explore the utility of eDNA based methods for validating other reported FSAs. Cross-utlisation of survey techniques and strengthening technical capacity for FSA monitoring teams through standardised protocols will be important moving forward.

Social science and advocacy

Despite best efforts, representation from conservation and enforcement stakeholders was limited in the questionnaire analysis, necessitating the need for additional follow-up with these groups, with particular emphasis on enforcement stakeholders. Future assessments of both fishers and consumers are also required as both stakeholder groups significantly impact the resource (i.e. through harvesting, purchasing, consumption and disturbances to Nassau grouper habitats). Partnerships with social science experts are a logical progression for

advancing this work and determining the best methods for reducing knowledge gaps and increasing compliance for established fishery regulations.

Reproductive physiology

Several hypotheses have been proposed to explain how and why Nassau grouper utilise FSAs as a reproductive strategy. These include: increasing reproductive output, minimising predation risk on adults/larvae, and habitat/resource availability (reviewed by Molloy et al. 2012). Limited progress, however, has been made in determining which of these hypotheses are correct, but correlates to the lunar cycle and water temperature have been documented (Colin 1992). Nonetheless, determining the impacts of overfishing on the reproductive potential or success of the species remains an important priority. This will require: 1) quantifying the reproductive output of active FSAs, 2) identifying hormones responsible for sex determination and ovarian development to better establish reproductive endocrinology of Nassau grouper, 3) investigating intraspecific differences in fecundity across FSAs, 4) examining the relationship between growth, sexual development and overall fitness with genetic variation across the archipelago, and 5) comparisons of physiological parameters of spawning and non-spawning Nassau grouper to better understand the physiological costs associated with FSA reproduction. Finally, as a gonochoristic species, developing a biomarker for sex determination would be useful to assist with monitoring population demographics.

Advancing Conservation Management for Nassau Grouper

Assessing the capacity of fish species to withstand exploitation when faced with stochastic natural and anthropogenic stressors is of critical importance for effective fisheries management. A number of methods exist, spanning diverse fields of biological and social science to understand how fish species are likely to withstand these stressors (Selkoe et al. 2016; Shafer et al. 2016; Paris et al. 2018). For fisheries management, traditionally, stock-recruitment models have been used

to estimate maximum sustainable yield and to define harvest rates or quotas (Hilborn and Walters, 1992). Such models account for natural and fishing mortality, growth rates and recruitment, and larval dispersal modelling, but were beyond the scope of this research and should be undertaken.

Costello et al. (2012) estimated that improving the state of global fisheries, can lead to increases in fish yields (up to 40 %) and stock abundance (~56 %), both of which have important implications for food and economic security. Globally, varied approaches have been used for species conservation including fishery regulations (e.g., gear restrictions, harvest quotas, size limits and closed seasons), fishing cooperatives, no-take marine protected areas (MPAs), MPA networks, catch shares, territorial user right fisheries (TURFs), and multi-species and species-specific management plans (Hilborn 2007; Turner et al. 2014; Costello et al. 2012); moreover, there are limitations to each approach. Failure to use scientific data as the foundation for any of these approaches has been shown to reduce the successfulness of desired management outcomes. For example, for migrating species like Nassau grouper, not only is age at sexual maturity an important determinant for establishing minimum size limits, but also the average size at which fish make their first migrations to spawning sites (Dahlgren et al. 2016; Sherman et al. 2016). MPAs like the Exuma Cays Land and Sea Park (ECLSP) that do not account for connectivity and/or have not been explicitly designed to address fisheries management objectives are also unlikely to be completely effective conservation management tools (Sherman et al. In Press; Chapter IV). Nonetheless, science-based regulations that incorporate stakeholder perspectives still represent a holistic approach that can be used to guide the development of management plans (Appendix I: Sherman et al. 2018).

Species management or recovery plans, however, have often been criticized for inadequately incorporating scientific data and providing biological justifications for proposed recovery actions (Doak et al. 2015). Aside from financial constraints, successful implementation of management plans can be limited by socio-political (e.g. political will) and cultural factors, and failure to create incentives for key stakeholders (Taylor et al. 2005; Hilborn 2007). In an analysis of more than 1,000 threatened and endangered species on the Endangered Species Act (ESA),

Taylor et al. (2005) found that early recognition of at-risk species along with the implementation of well-funded species-specific management plans, which accounted for their life history characteristics and associated habitat requirements, helped to better facilitate recovery compared with those that did not. Despite more than 20 years of being classified as endangered on the IUCN, to my knowledge, no management plans have been created for Nassau grouper prior to the conservation plan developed for The Bahamas (Appendix I: Sherman et al. 2018) in this study.

The proposed management plan was designed to stimulate population recovery and to promote sustainability of the Bahamian Nassau grouper fishery. Four specific objectives were outlined in the plan including 1) increasing density and spawning stock biomass of Nassau grouper, 2) establishing sustainable harvest regulations, 3) reducing anthropogenic threats to the species and 4) maintaining and/ or improving critical marine habitats. Following recommendations by Doak et al. (2015), scientific justifications for management strategies were provided along with suggested recovery actions and criteria to evaluate the efficacy of these measures. In addition to regulatory amendments, managing authorities and relevant conservation groups were encouraged to collaboratively address national compliance issues that hinder population recovery efforts (Appendix I: Sherman et al. 2018).

Final remarks

The combination of microsatellite analysis, RAD-seq, acoustic telemetry and FSA surveys, were useful techniques that enabled a deeper understanding of Bahamian Nassau grouper through empirical investigations of genetic population dynamics, migration patterns, spawning stock biomass, and spawning behaviour. However, establishing linkages between the reproductive, physiological, ecological and environmental conditions experienced by Nassau grouper through ontogeny remains an important challenge for its conservation. Critical reviews of fisheries governance and stakeholder assessments helped to explain some of the existing challenges of managing this culturally, economically and ecologically important species within The Bahamas. More importantly, the outputs were useful in

providing appropriate science-grounded recommendations and identifying preliminary stakeholder support for potential management directives that have been communicated to the Department of Marine Resources. While the research presented in this thesis will help to facilitate management and possibly policy changes for Nassau grouper conservation in The Bahamas, the approaches used are also applicable throughout the native range of the species, enabling regional comparisons to develop a holistic understanding of spatial ecology, connectivity and reproductive biology to better inform conservation management practises for this critically endangered species.

References

Baran R, Jůza T, Tušer M, Balk H, Blabolil P, Čech M, Draštík V, Frouzová, J, Jayasinghe AD, Koliada I, Mrkvička T, Muška M, Ricard D, Sajdlová Z, Vejřík L, Kubečka J (2017) A novel upward-looking hydroacoustic method for improving pelagic fish surveys. Nature Scientific Reports 7:4823, doi: 10.1038/s41598-017-04953-6

Bekkevold D, Helyar SJ, Limborg MT, Nielsen EE, Hemmer-Hansen J, Clausen LA, Carvalho GR (2015) Gene-associated markers can assign origin in a weakly structured fish, Atlantic herring. ICES Journal of Marine Science 72:1790–1801

Bourret V, Dionne M, Kent MP, Lien S, Bernatchez L (2013) Landscape genomics in Atlantic salmon (*Salmo salar*): searching for gene-environment interactions driving local adaptation. Evolution 67:3469−3487

Bradbury IR, Hamilton LC, Chaput G, Robertson MJ, Goraguer H, Walsh A, Morris V, Reddin D, Dempson JB, Sheehan TF, King T, Bernatchez L (2016) Genetic mixed stock analysis of an interceptory Atlantic salmon fishery in the Northwest Atlantic. Fisheries Research 174: 234−244

Bylemans J, Furlan EM, Pearce L, Daly T, Gleeson DM (2016) Improving the containment of a freshwater invader using environmental DNA (eDNA) based monitoring. Biological Invasions doi:10.1007/s10530-016-1203-5

Bylemans J, Furlan EM, Hardy CM, McGuffie P, Lintermans M, Gleeson DM (2017) An environmental DNA (eDNA) based method for monitoring spawning activity: a case study, using the endangered Macquarie perch (*Macquaria australasica*). Methods in Ecology and Evolution 8:646−655

Colin PL (1992) Reproduction of the Nassau grouper, *Epinephelus striatus* (Pisces: Serranidae) and its relationship to environmental conditions. Environmental Biology of Fishes 34:357−377

Delmore KE, Hübner S, Kane NC, Schuster R, Andrew RL, Câmara F, Guigó R, Irwin DE (2015) Genomic analysis of a migratory divide reveals candidate genes for migration and implicates selective sweeps in generating islands of differentiation. Molecular Ecology 8:1873−1888

Doak DF, Himes Boor GK, Bakker VJ, Morris WF, Louthan A, Morrison SA, Stanley A, Crowder LB (2015) Recommendations for improving recovery criteria under the US Endangered Species Act. BioScience 65:189−199

Drinan DP, Galindo HM, Loher T, Hauser L (2016) Subtle genetic population structure in Pacific halibut *Hippoglossus stenolepis*. Journal of Fish Biology 89:2571−2594

Egerton JP, Johnson AF, Le Vay L, McCoy CM, Semmens BX, Heppell SA, Turner JR (2017) Hydroacoustics for the discovery and quantification of Nassau grouper (*Epinephelus striatus*) spawning aggregations. Coral Reefs 36:589−600

Ehrhardt NM, Deleveaux VKW (2007) The Bahamas' Nassau grouper (*Epinephelus striatus*) fishery—two assessment methods applied to a data-deficient coastal population. Fisheries Research 87:17−27

Excoffier L, Dupanloup I, Huerta-Sánchez E, Foll M (2013) Robust demographic inference from genomic and SNP data. PLOS Genetics 9:e1003905

Excoffier L, Foll M (2011) fastsimcoal: a continuous-time coalescent simulator of genomic diversity under arbitrarily complex evolutionary scenarios. Bioinformatics 27:1332−1334

Fraser K, Rusello M (2013) Lack of parallel genetic patterns underlying the repeated ecological divergence of beach and stream spawning kokanee salmon. Journal of Evolutionary Biology 26:2606−2621

Haasl RJ, Payseur BA (2011) Multi-locus inference of population structure: a comparison between single nucleotide polymorphisms and microsatellites. Heredity 06:158–171

Harrison HB, Williamson DH, Evans RD, Almany GR, Thorrold SR, Russ GR, Feldheim KA, van Herwerden L, Planes S, Srinivasan M, Berumen ML, Jones GP (2012) Larval export from marine reserves and the recruitment benefit for fish and fisheries. Current Biology 22:1023–1028

Heppell SA, Semmens BX, Archer SK, Pattengill-Semmens CV and others (2012) Documenting recovery of a spawning aggregation through size frequency analysis from underwater laser callipers measurements. Biological Conservation 155:119−127

Hilborn R, Orensanz JM, Parma AM (2005) Institutions, incentives and the future of fisheries. Philosophical Transactions of the Royal Society B Biological Sciences 360:47−57

Lacoursière-Roussel A, Rosabal M, Bernatchez L (2016) Estimating fish abundance and biomass from eDNA concentrations: variability among capture methods and environ- mental conditions. Molecular Ecology Resources 16:1401–1414

Liu N, Chen L, Wang S, Oh C, Zhao H (2005) Comparison of single-nucleotide polymorphisms and microsatellites in inference of population structure. BMC Genetics 6(Suppl 1)**:**S26 doi:10.1186/1471-2156-6-S1-S26

Mascolino S, Benvenuto C, Gubili C, Sacchi C, Boufana B, Mariani S (2016) The ART of mating: alternative reproductive tactics and mating success in a nest-guarding fish. Journal of Fish Biology 89:2643–2657

McMahon BJ, Teeling EC, Höglund J (2014) How and why should we implement genomics into conservation? Evolutionary Applications doi:10.1111/eva.12193

Molloy PP, Côté IM, Reynolds JD (2012) Why spawn in aggregations? In: Sadovy de Mitcheson Y, Colin PL (eds) Reef fish spawning aggregations: biology, research and management, Springer, New York, NY, p 57−84

Moore J-S, Harris LN, Le Luyer, J, Sutherland BJG, Rougemont Q, Tallman RF, Fisk AT, Bernatchez L (2017) Migration harshness drives habitat choice and local adaptation in anadromous Arctic char: evidence from integrating population genomics and acoustic telemetry. bioRxiv doi:10.1101/138545

Paris JR, Sherman KD, Bell E, Boulenger C, Delord C, El-Mahdi MBM, Fairfield EA, Griffiths AM, Gutmann Roberts C, Hedger RD, Holman LE, Hooper LH, Humphries NE, Katsiadaki I, King RA, Lemopoulos A, Payne CJ, Peirson G, Richter KK, Taylor MI, Trueman CN, Hayden B, Stevens JR (2018) Understanding and managing fish populations: keeping the toolbox fit for purpose. Journal of Fish Biology 92:727–751, doi:10.1111/jfb.13549

Putman AI and Carbone I (2014) Challenges in analysis and interpretation of microsatellite data for population genetic studies Ecology and Evolution 4:4399–4428

Sakata MK, Maki N, Sugiyama H, Minamoto T (2017) Identifying a breeding habitat of a critically endangered fish, *Acheilognathus typus*, in a natural river in Japan. The Science of Nature 104:100

Schrider DR, Kern AD (2018) Supervised machine learning for population genetics: a new paradigm. Trends in Genetics doi:10.1016/j.tig.2017.12.005

Selkoe KA, Blenckner T, Caldwell MR, Crowder LB, Erickson AL, Essington TE, Estes JA, Fujita RM, Halpern BS, Hunsicker ME, Kappel CV, Kelly RP, Kittinger JN, Levin PS, Lynham JM, Mach ME, Martone RG, Mease LA, Salomon AK, Samhouri JF, Scarborough C, Stier AC, White C, Zedler J (2016) Principles for managing marine ecosystems prone to tipping points. Ecosystem Health and Sustainability 1:17 doi: 10.1890/EHS14-0024.1

Shafer ABA, Northrup JM, Wikelski M, Wittemyer G, Wolf JBW (2016) Forecasting Ecological Genomics: High-Tech Animal Instrumentation Meets High-Throughput Sequencing. PLoS Biology 14:e1002350, doi:10.1371/journal.pbio.1002350

Sherman KD, Dahlgren CP, Stevens JR, Tyler CR (2016) Integrating population biology into conservation management for endangered Nassau grouper *Epinephelus striatus* Marine Ecology Progress Series 554:263−280

Sherman KD, Dahlgren CP, Knowles LC (2018) Nassau Grouper (*Epinephelus striatus*) Conservation Management Plan for The Commonwealth of The Bahamas. Prepared for the Department of Marine Resources, Nassau, Bahamas.

Sherman KD, King RA, Dahlgren CP, Simpson SD, Steven JR, Tyler CR (2017) Historic and contemporary anthropogenic activities influence genetic population dynamics of Nassau grouper (*Epinephelus striatus*) within The Bahamas. Frontiers in Marine Science 4:393, doi:10.3389/fmars.2017.00393

Sherman KD, Shultz AD, Dahlgren CP, Thomas C, Brooks E, Brooks A, Brumbaugh DR, Gittens L, Murchie K (In Press) Contemporary and emerging fisheries in The Bahamas – conservation and management challenges, achievements and future directions. Fisheries Management and Ecology, doi: 10.1111/fme.12299

Stump K, Dahlgren CP, Sherman KD, Knapp C. (2017) Nassau grouper migrations during full moon suggest collapsed historic fish spawning aggregation and evidence of undocumented aggregation. Bulletin of Marine Science 93:375–389

Sylvester EVA, Bentzen P, Bradbury IR, Clément M, Pearce J, Horne J, Beiko RG (2017) Applications of random forest feature selection for fine-scale genetic population assignment. Evolutionary Applications 11:153–165

Taylor MFJ, Suckling F, Rachlinski JJ (2005) The effectiveness of the Endangered Species Act: a quantitative analysis. BioScience 55:360−367

Turner RA, Polunin NVC, Stead SM (2014) Social networks and fishers' behaviour: exploring the links between information flow and fishing success in the Northumberland lobster fishery. Ecology and Society 19:38, doi:10.5751/ES-06456-190238

Yu H, You X, Li J, Liu H, Meng Z, Xiao L, Zhang H, Lin H-R, Zhang Y, Shi Q (2016) Genome-wide mapping of growth-related quantitative trait loci in orange-spotted grouper (*Epinephelus coioides*) using double digest restriction-site associated dNA sequencing (ddRADseq). International Journal of Molecular Sciences 17:501, doi:10.3390/ijms17040501

Appendices

Appendix I: Nassau Grouper (*Epinephelus striatus*) conservation management plan for The Commonwealth of The Bahamas

Technical Document Submitted to the Department of Marine Resources

Authors: Sherman KD, Dahlgren CP and Knowles LC

Nassau Grouper (*Epinephelus striatus*) Conservation Management Plan

for

The Commonwealth of The Bahamas

Submitted to the: Department of Marine Resources

Nassau, Bahamas

Photography

Craig Dahlgren
Shane Gross
Charles Knapp
Keith Pamper
Krista Sherman
Aaron Shultz

Cover Design: Krista Sherman

Nassau grouper Illustrations: Jonathan Cartwright

Nassau grouper FSA, bathymetry and habitat maps: Lindy Knowles

Citation

Sherman KD, Dahlgren CP, Knowles LC (2018) Nassau Grouper (*Epinephelus striatus*) Conservation Management Plan for The Commonwealth of The Bahamas. Prepared for the Department of Marine Resources, Nassau, Bahamas.

Available from:
Department of Marine Resources
Ministry of Agriculture, Marine Resources and Local Government
East Bay Street
P. O. Box N-3028
Nassau, Bahamas
Tel. (242) 393-1777
Fax (242) 393-0238

Department of Marine Resources

The Commonwealth of The Bahamas

List of Abbreviations

AGRRA	Atlantic Gulf Rapid Reef Assessment
BNT	Bahamas National Trust
BCFA	Bahamas Commercial Fishers Alliance
BNPAS	Bahamas National Protected Area System
BPAF	Bahamas Protected Area Fund
BREEF	Bahamas Reef Environment Educational Foundation
CBD	Convention on Biological Diversity
CEI	Cape Eleuthera Institute
CFMC	Caribbean Fishery Management Council
CMU	Conservation Management Unit
CPUE	Catch Per Unit Effort
CRFM	Caribbean Regional Fisheries Mechanism
DMR	Department of Marine Resources
EEZ	Exclusive Economic Zone
ESA	Endangered Species Act
FAO	Food and Agriculture Organization
FSA	Fish Spawning Aggregation
GCFI	Gulf and Caribbean Fisheries Institute
GEF	Global Environment Facility
IAS	Invasive Alien Species
IUCN	World Conservation Union (formerly International Union for Conservation of Nature)
IUU	Illegal Unreported and Unregulated Fishing
MPA	Marine Protected Area
MSY	Maximum Sustainable Yield
NGO	Non-Governmental Organization
NISP	National Implementation Support Programme
NISS	National Invasive Species Strategy
NOAA	National Oceanic and Atmospheric Administration
POWPA	Programme of Work on Protected Areas
RTD	Disney Conservation Fund's Reverse The Decline Program
SOSF	Save Our Seas Foundation
SNP	Single nucleotide polymorphism
SPAW	Special Protected Areas and Wildlife
TOTO	Tongue of the Ocean
WECAFC	Western Central Atlantic Fisheries Commission

Executive Summary

Nassau grouper (*Epinephelus striatus*) are listed as a **critically endangered species** by the World Conservation Union (IUCN), with one of the last viable populations found in The Bahamas, where they are of significant cultural, ecological and economic value as a fishery species. Because Nassau grouper are slow growing and late maturing fish that migrate 300 km (186 mi) or more to spawn once each year in mass aggregations, they are easily overfished. Thus, balancing the conservation of a critically endangered species with maintaining an economically viable fishery requires a well-defined conservation and management strategy. Sustainable fisheries strategies are implemented to promote the maintenance of healthy stocks and biological functions of marine species and habitats while maximizing economic gain and ensuring food security. This management strategy discusses the current status of Nassau grouper stocks in The Bahamas and addresses threats to Nassau grouper stocks within the country to promote long-term sustainable use of the species.

Nassau grouper fisheries peaked in the 1990's with landings of 514 tonnes and a value of over $3 million making it the most productive and most valuable scalefish fishery in The Bahamas. Over the past 20 years however, landings have declined by 86% and the value of the fishery has decreased by two thirds. This decline is also evident in decreases in abundance within marine habitats, collapses of known historic spawning aggregations and noticeable losses in effective population size throughout The Bahamas. Of the 30-40 reported spawning aggregations in The Bahamas that have been reported, most remain unverified with no information on whether they still form. Those that have been assessed show that several historic Nassau grouper spawning aggregations no longer form. Similarly, while historical information from spawning aggregations indicates that aggregations of 10,000 to 100,000 fish was common, most of the remaining aggregations surveyed to date only support hundreds of spawning fish during peak periods, with only two having over 1,000 Nassau grouper aggregating at any time. Clearly, these declines indicate the need for more effective management to rebuild stocks and ensure sustainable fisheries.

The overall goal of this management plan is to promote population recovery and sustainability of the Bahamian Nassau grouper fishery.

Specific management objectives are to:

1. Increase Nassau grouper density and spawning stock biomass
2. Improve harvest regulations to promote sustainability of the fishery
3. Reduce anthropogenic threats
4. Maintain and/ or improve essential marine habitats.

At present, Nassau grouper in The Bahamas face threats from both natural and anthropogenic factors including high levels of fishing, habitat degradation, invasive alien species, disease and predation, and climate change. Of these threats, those related to fishing are the most severe and the ones that may be managed most effectively, but loss of juvenile nursery habitat in mangrove systems was also rated high as a threat. The threats posed by fishing come in the form of both illegal fishing (e.g., capture of fish below the legal size limit or during the closed season) and by fishing practices that may be within the scope of fisheries regulations, but are unsustainable due to the biology of the species (e.g., certain fishing gears that are legal, but threaten populations by landing legal sized fish that are still sexually immature or inexperienced spawners). The former requires strategies aimed at building compliance with fisheries regulations through education, enforcement and other means. The latter requires strategies to improve fisheries regulations based on science.

To address legal but unsustainable fishing practices the following actions are recommended that require amending current fishing regulations:

1. ***Extending the closed season from November 1 through March 31*** The current season is December 1-February 28. During years when the full moon falls early in December or the last week of November, fish may migrate to spawning aggregations before the start of the closed season. Similarly, when the full moon falls in late February, fish may still be at spawning aggregations after the season opens.
2. ***Increasing the minimum size limit to 54 cm TL (21.3 in.) or 4 kg (~9 lbs.)*** The current minimum size is 3 lbs. While fish may reach reproductive maturity as early as 3 lbs., 54 cm TL (roughly 4 kg or 9 lbs.) is the size at which >75% of the population is mature and the size at which fish migrate to spawning aggregations. The increase in minimum size limit would effectively prevent the capture of most immature fish before they can reproduce. Furthermore, we suggest including a minimum length in addition to weight to help fishers easily gauge size of fish at capture.
3. ***Banning the use of traps around FSAs during the spawning season*** There are no current restrictions on traps. While traps may be used to target a number of species, they are highly effective for capturing Nassau grouper at spawning aggregations. Furthermore, traps that are lost may continue to capture and kill fish indiscriminately as ghost traps. To prevent capture of Nassau grouper as bycatch at spawning sites and to prevent ghost traps at spawning sites, a ban on fish traps in these areas should be implemented during the spawning season.
4. ***Protect multi-species fish spawning aggregations*** No protection of multi-species aggregations exists at present. Because many species of grouper and snapper use the same spawning sites as Nassau grouper at other times of the year, and face the same threats of fishing at spawning times that Nassau grouper do, establishing protection for these sites is recommended.
5. ***Establishment of a maximum size limit*** There is currently no maximum size limit. Because larger females contribute disproportionately to reproductive output due to their high fecundity, the possibility of protecting these larger fish should be considered.

In addition to these recommendations, it is also recommended to further support ongoing FSA monitoring and stock assessment efforts, enforce existing regulations through improved strategic surveillance during the closed season, including patrols of spawning sites, inspections at points of sale and other parts of the supply chain such as fish houses or mailboats, increasing fines for illegal activities, enlisting help of fishers in reporting illegal activity and publishing fines associated with illegal fishing activity. Furthermore, incorporation of priority spawning sites into marine protected areas as part of the expansion of the Bahamas National Protected Area System is also recommended to help with enforcement.

Finally, a framework for periodic evaluation of management effectiveness and adaptation of the management plan is recommended to ensure that specific goals or targets are being met to promote recovery of Nassau grouper stocks and ensure sustainable fishing practices are used to preserve this fishery for generations of Bahamians.

Table of Contents

List of Figures

List of Tables

Introduction

Overview

The Nassau grouper is an ecologically, economically and culturally valuable species that is exploited by commercial, recreational and subsistence fisheries in The Bahamas and throughout the Caribbean. In 1996, the World Conservation Union (IUCN) classified Nassau grouper as endangered (EN A2ad) on the Red List of Threatened Species (Cornish and Eklund 2003). Global efforts to rebuild Nassau grouper populations have included complete moratoriums, seasonal closures, size limits, the implementation of marine protected areas (MPAs), quotas and fish spawning aggregation (FSA) closures and FSA protected areas (Sadovy de Mitcheson et al. 2013). However, anthropogenic impacts persist, and significant declines of 60% or more have been documented worldwide along with the disappearance of 33% of historic FSAs (Sadovy de Mitcheson et al. 2008; Sadovy de Mitcheson and Erisman 2012). More recently, worsening trends in population abundance led to Nassau grouper being re-classified as a critically endangered (CR A2bd) species by the IUCN (Carpenter et al. 2015; http://www.iucnredlist.org/details/7862/25) and listed as a threatened species on the United States Endangered Species Act (Federal Register 2016). In The Bahamas, declining fish stock biomass and FSAs threaten the long-term sustainability of the Nassau grouper fishery (Sherman et al. 2016; Stump et al. 2017; Sherman et al. 2017) and Chueng et al. (2013) have suggested that the fishery has already been overexploited. To address this, we have developed a scientifically-based sustainable conservation management plan for Nassau grouper in consultation with experts and key stakeholders. The purpose of this conservation management strategy is to:

1. Describe and summarize the coordinated efforts required to sustainably manage Nassau grouper populations and its critical habitats in The Bahamas;
2. Specify the population(s), habitat, and harvest regulations to maintain recovered (i.e. abundant, reproductively viable and genetically diverse) Nassau grouper populations;
3. Explain the regulatory mechanisms, legal authorities, policies, management, and monitoring programs that exist to manage Nassau grouper in The Bahamas.
4. Document the individuals and agencies committed to the restoration of Nassau grouper populations and the sustainable management of the commercial fishery in The Bahamas.

This management plan is designed to be adaptive and its implementation should facilitate recovery of Nassau grouper populations and promote sustainable harvest of the commercial fishery in The Bahamas.

Species Description & Taxonomic Classification

Photo 1. Various colour phases adopted by Nassau grouper: normal or barred, bicolour, white belly and dark.
Photo credits: top (l-r) - Keith Pamper, Krista Sherman, bottom (l-r) Shane Gross, Charles Knapp

Bloch (1792) first described the species now commonly known as Nassau grouper, *Epinephelus striatus*. Scales are ctenoid and fish possess an opercula spine, 11-12 dorsal spines, 16-18 dorsal fin rays, 3 anal spines and 8 anal rays (Heemstra and Randall 1993; Sadovy and Eklund 1999; Froese and Pauly 2016). Normal skin colouration is light grey or olive to reddish brown (Photo 1 – top l). Distinguishing features include five dark bars along the body, a tuning fork shaped pattern on the head, a black blotch on the caudal peduncle and a series of black dots posterior to or below the eye. Territorial, mating and other behaviours can result in rapid colour and pattern changes including bicolour, white belly and dark phase (Photo 1; Heemstra and Randall 1993; Archer et al. 2012; Watson et al. 2014). Variations of these colour phases and patterns have also been documented (e.g. Colin 1992; Whaylen et al. 2007; Watson et al. 2014; Photo 2).

Although previously classified as a member of the seabass Family, Serranidae (Heemstra and Randall 1993), more recent taxonomic classifications place the species under the Family Epinephelidae (Craig et al. 2011; Ma et al. 2016). The largest Nassau grouper on record (122 cm TL; 27 kg) was from Puerto Rico (Sadovy and Eklund 1999), but adults in fished areas typically range between 55-70 cm TL (Bush et al. 2006).

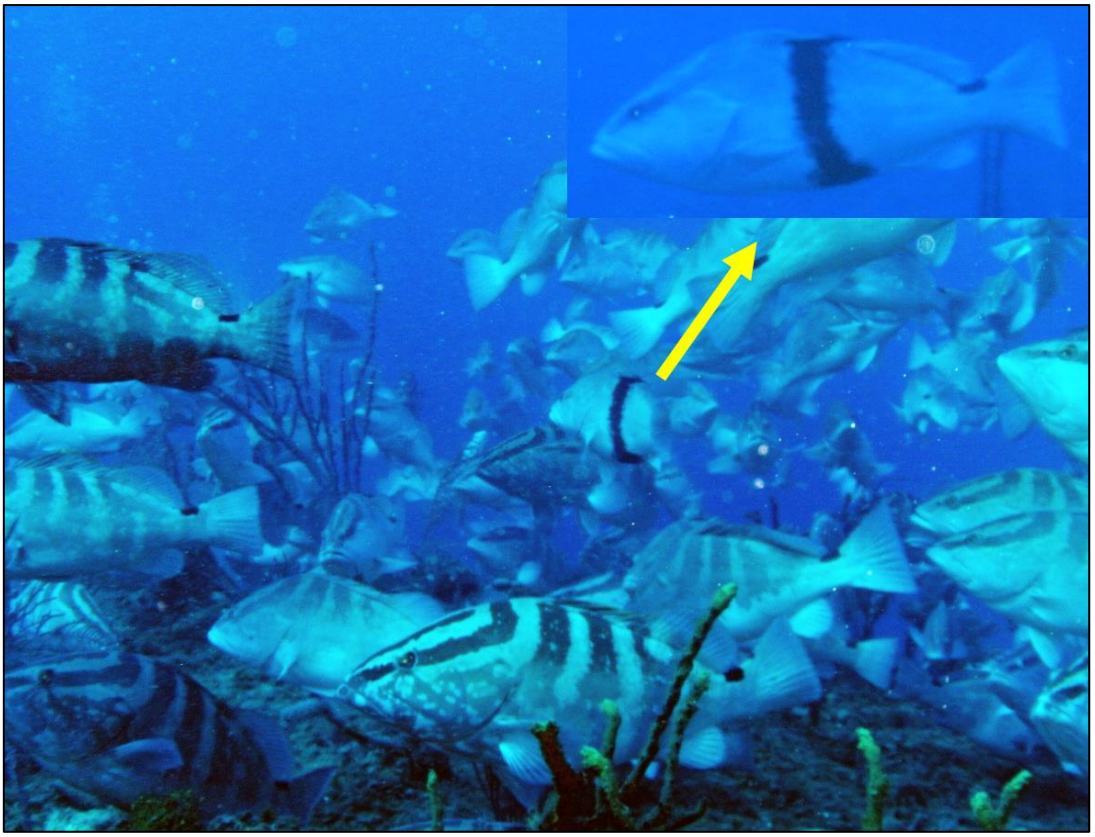


Photo 2. Aberrant colour phase of a Nassau grouper observed at the Hail Mary spawning aggregation site off Long Island during December 2013.
Photo credits: Krista Sherman

Taxonomy

Kingdom Animalia
Phylum Chordata
Class Actinopterygii
Order Perciformes
Family Epinephelidae
Subfamily Epinepheinae
Genus *Epinephelus*
Species *striatus*

Geographic Distribution & Habitat Use

Nassau grouper inhabit insular marine habitats (i.e. mangroves, seagrasses, hardbottom and coral reefs), with a maximum reported depth of 255 m (Starr et al. 2007). Their natural distribution includes the Tropical Western Atlantic including Bermuda, Florida, The Bahamas and Yucatan Peninsula, the Caribbean Sea and parts of the Gulf of Mexico (Heemstra and Randall 1993; Albins et al. 2009; Froese and Pauly 2016; Fig. 1).

Figure 1. Global distribution map of Nassau grouper, *Epinephelus striatus*. Image created by Jack Cook, Woods Hole Oceanographic Institute Graphic Services.

As an archipelagic nation, The Bahamas possesses large stretches (~260,000 km^2) of potentially suitable marine habitats for Nassau grouper (Figs. 2, 3, 4). Newly settled recruits are strongly associated with small colonies of *Porites* sp., macroalage and seagrass – particularly *Laurencia* spp. and *Thalassia testudinum* (Sadovy and Eklund 1999). Juveniles and subadults are more prevalent in shallow water, inhabiting microhabitats within nursery areas and patch reefs (Eggleston 1995; Eggleston et al. 1998; Grover et al. 1998; Dahlgren end Eggleston 2001; Dahlgren et al. 2006; Camp et al. 2013). Adults tend to establish home ranges (0.1-0.2 km) in more rugose coral reefs, hardbottom habitats, or other high-relief structures (Bolden 2000).

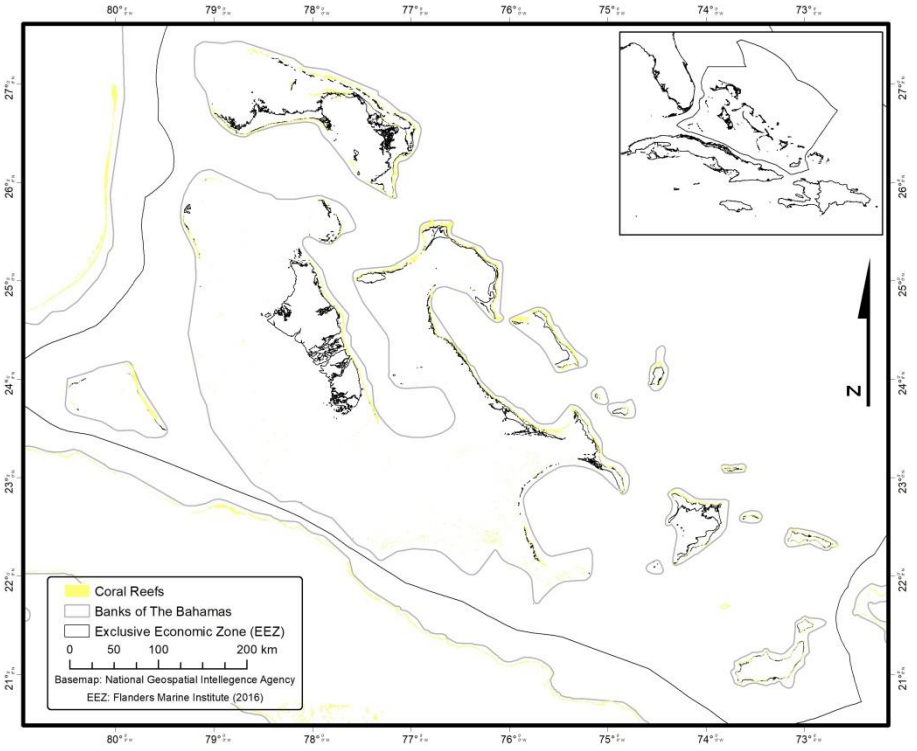


Figure 2. GIS map of coral reef habitats in The Bahamas.
Map: *Lindy Knowles*

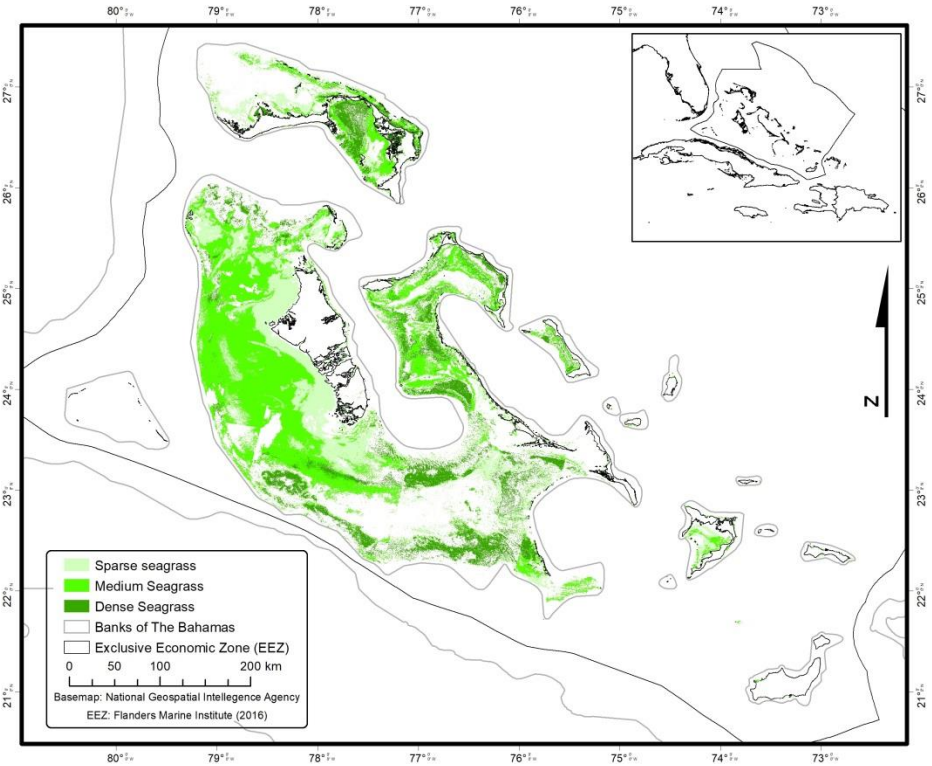


Figure 3. GIS habitat map of seagrasses (by density) in The Bahamas.
Map: *Lindy Knowles*

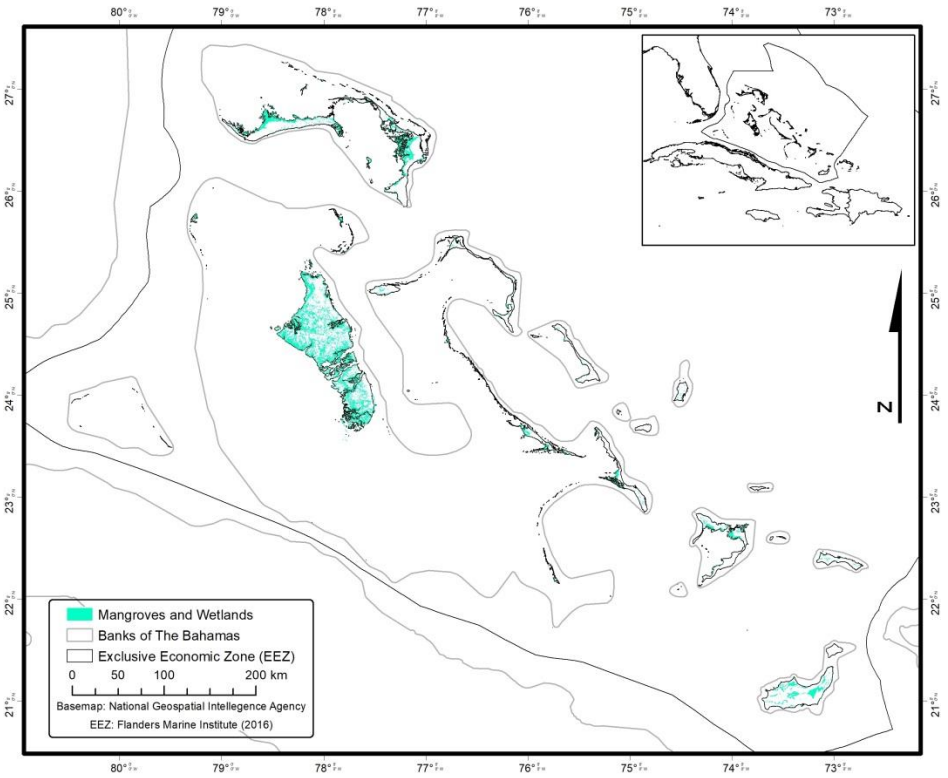


Figure 4. GIS habitat map of mangroves and wetlands in The Bahamas.
Map: *Lindy Knowles*

Ecology

Zooplankton and copepods are the primary food source for pelagic juveniles ranging between 22-27 mm SL (Colin 1992; Grover 1993; Colin et al. 1997; Grover et al. 1998). Following recruitment to appropriate macroalgal habitats as demersal juveniles (25-35 mm TL), Nassau grouper undergo a series of ontogenetic dietary and associated habitat shifts (Dahlgren and Eggleston 2001; Dahlgren et al. 2014). Nassau grouper are important generalist predators within the marine environment, regulating ecosystem dynamics through the consumption of lower trophic level species. Their diet is comprised of a diverse range of both invertebrates and vertebrates (reviewed by Sadovy and Eklund 1999), although adults are mostly piscivores (>50% fish; Heemstra and Randall 1993) as evidenced by stomach content and stable isotope analyses (Appendix A; Carter et al. 1994; Eggleston et al. 1998; O'Farrell et al. 2014). Using ambush feeding tactics, Nassau grouper consume the majority of their prey during crepuscular hours (Sadovy and Eklund 1999).

Reproduction & Life History

Nassau grouper are gonochoristic (i.e. they have separate sexes), oviparous pelagic spawners (Sadovy and Colin 1995), reaching sexual maturity between the ages of 4-8 years (≥48 cm TL) (Sadovy and Eklund 1999; Froese and Pauly 2016), as evidenced by the presence of mature vitellogenic or hydrated oocytes in the ovaries and spermatozoa and spermatids in the testis (Sadovy and Colin 1995; Cushion et al. 2008). Nassau grouper gonads are bilobate, possessing a llamellar structure and lumen (Carter et al. 1991; Sadovy and Colin 1995). Fish are classified as immature to mature based on stages of ovarian and testicular development including the presence of primary oocytes, cortical alveoli, vitellogenic oocytes and hydrated oocytes in females; and the presence of spermatogonia, spermatocytes, spermatocysts and spermatozoa in males (Cushion et al. 2008).

Reproduction occurs at annual spawning events in synchrony with the lunar cycle and is also associated with cooler water temperatures (Colin 1992; Table 1). Histology, telemetry studies and spawning surveys have provided evidence to support that peak spawning in The Bahamas occurs within a few days around the full moon during the months of December and January (Colin 1992; Sadvoy and Colin 1995; Cushion et al. 2008; Dahlgren et al. 2016a). Hundreds to thousands of adults migrate 25 - >300 km to and from resident home reefs to spawn at masse gatherings with conspecifics (Smith 1972; Colin 1992; Bolden 2000; Dahlgren et al. 2016a; Stump et al. 2017) at specific locations. Around 30-40 Nassau grouper FSAs have been reported in The Bahamas and 60-80 have been reported worldwide (Fig. 5).

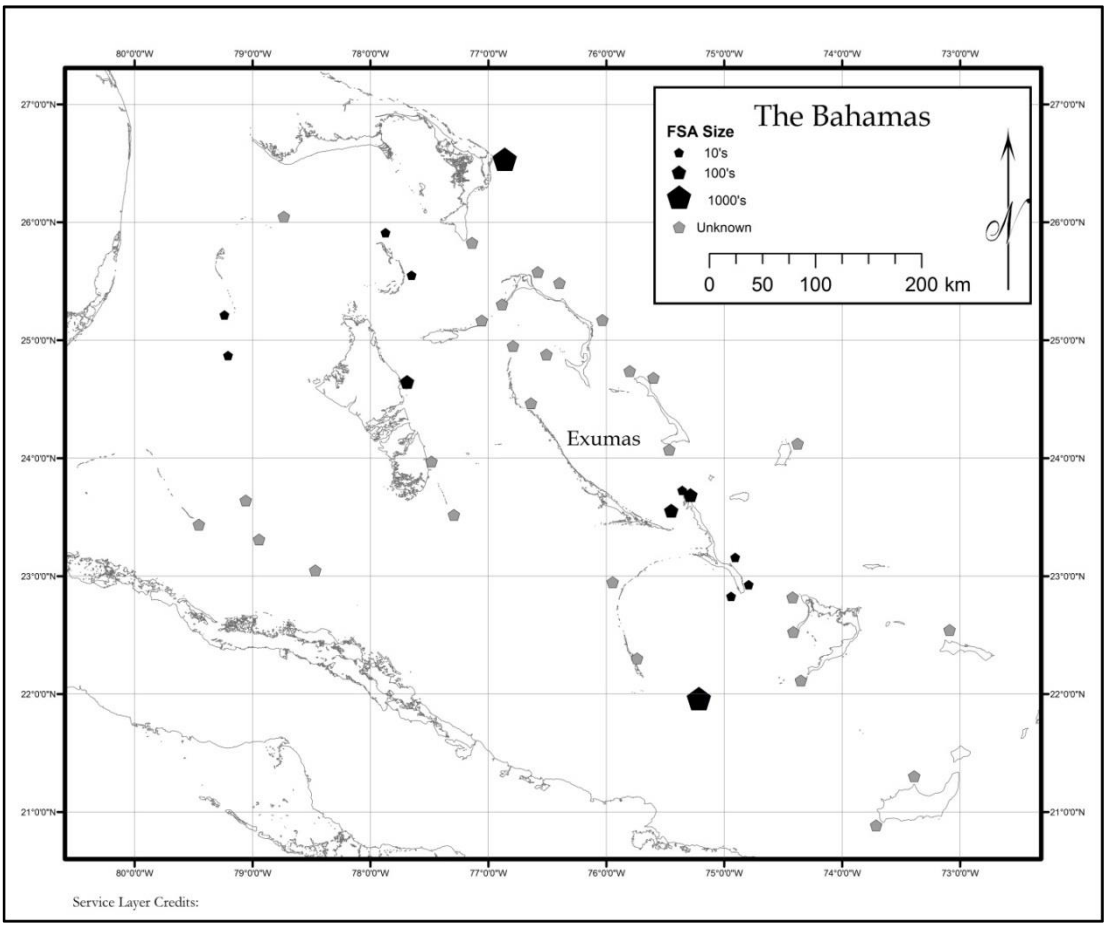


Figure 5. Map of reported Nassau grouper FSAs in The Bahamas (Sherman et al. 2016).

Courtship and mating behaviours are elaborate and complex, increasing in frequency and intensity leading up to and on the night of the full moon (Colin 1992). Readiness to reproduce is typically signalled by a noticeable shift from normal or barred coloration to bicolour phase (Colin 1992; Archer et al. 2012). Spawning rushes and gamete release usually occur one hour before and within 10-15 min after sunset (Colin 1992; Whaylen et al. 2004; Whaylen et al. 2007). Females are highly fecund, releasing millions of buoyant eggs (~ 1mm in diameter), which are fertilized and dispersed with water currents (Colin 1992; Sadovy and Eklund 1999; Fig. 6). Embryos hatch approximately 24 to 40 hours post fertilization (Powell and Tucker 1992). Pelagic larval development occurs over a 37-45 day period, with a mean pelagic larval dispersal (PLD) of 42 days (Colin et al. 1997; Fig. 6). Shenker (1993) hypothesized that wind-driven currents across continental shelves deliver larvae to appropriate settlement areas, which may help explain the episodic nature of recruitment.

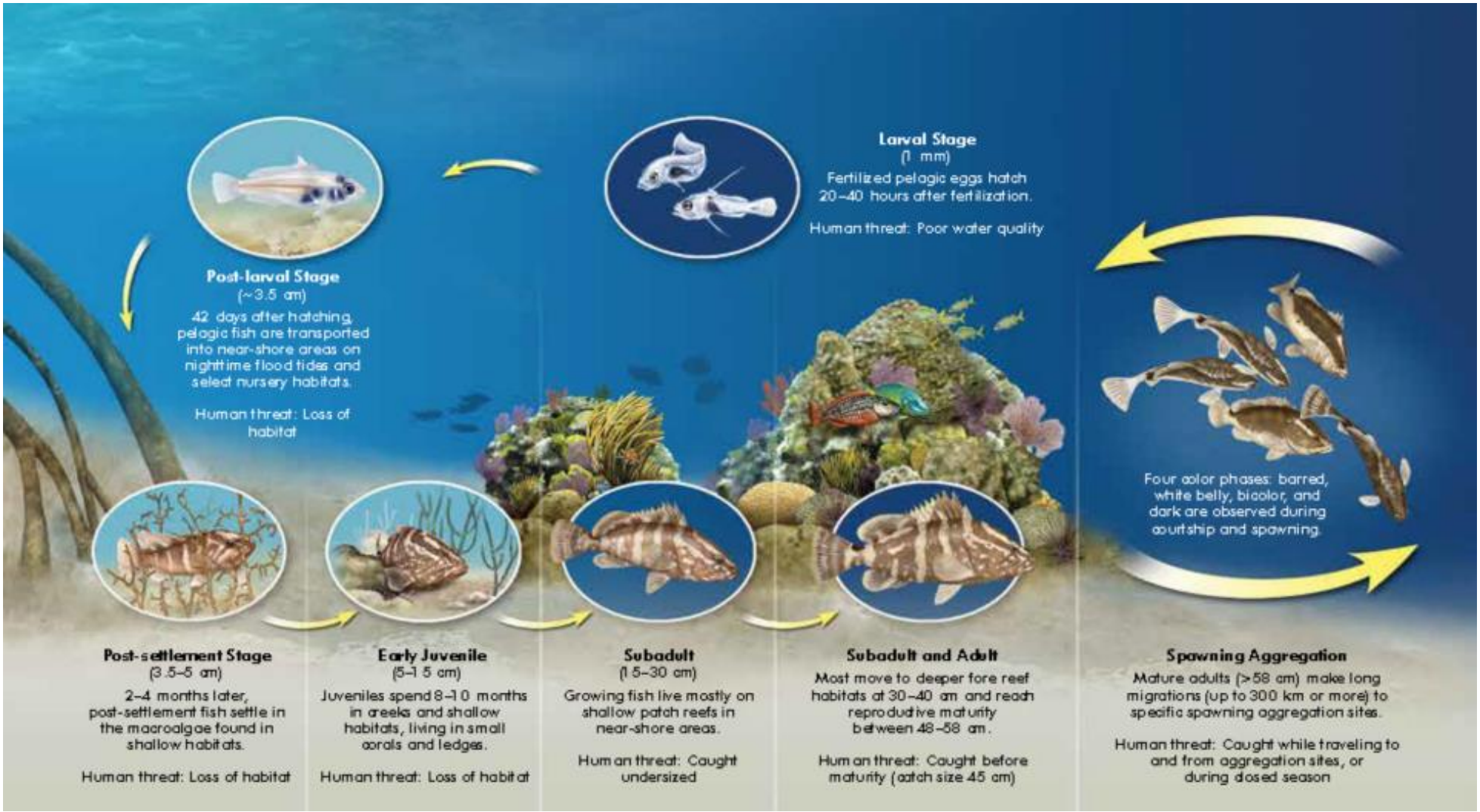


Figure 6. Life history, ontogenetic habitat requirements and associated anthropogenic threats to each life stage of Nassau grouper in The Bahamas. Graphic taken from the New Providence and Rose Island Coral Reef Report Card (Dahlgren et al. 2014).

Table 1. Intraspecific differences in Nassau grouper spawning occurrence throughout The Bahamas and Caribbean.

Location	Spawning Season	References
Belize	Full moon (December - March)	Carter et al. 1991; Heyman and Kjerfve 2008
Bermuda	Full moon (May - August)	Aguilar-Perera and Aguilar-Davila 1996; Whaylen et al. 2007
Cayman Islands	Full moon (December - March)	Whaylen et al. 2004; Whaylen et al. 2007
Cuba	Full moon or between the full and new moon (December - February)	Sadovy and Eklund 1999; Claro and Lindeman 2003
Jamaica	Full moon (January - April)	Thompson and Munro 1978
Honduras	Full moon (December - March)	Canty and Box 2013
Mexico	Full moon (December - January)	Aguilar-Perera et al. 2008
Puerto Rico	Full moon or between the full and new moon (January - May)	Schärer et al. 2010; Schärer et al. 2012; Schärer-Umpierre et al. 2014
The Bahamas	Full moon (November - March)	Smith 1972; Colin 1992; Ray 2000; Cushion and Sullivan-Sealey 2008; Dahlgren et al. 2016; Stump et al. 2017
U. S. Virgin Islands	Full moon or between the full and new moon (January - May)	Olsen and LaPlace 1978; Nemeth et al. 2006; Kadison et al. 2010

Age and Growth

Fertilized larvae grow up to 3.0 cm TL while in the planktonic phase (Shenker et al. 1993; Colin 1992; Colin et al. 1997; Fig. 6). Juvenile growth rates have been calculated based on otolith analysis and measurements of individual fish captured from both field and laboratory studies. Juveniles grow quickly, averaging 10 mm/month (Eggleston 1995; Photo 3). Adult growth and natural mortality rates vary throughout the species' native range (Sadovy and Eklund 1999). Sadovy and Colin (1995) estimated 21 years for the maximum age of Nassau grouper in The Bahamas, which is 8 years younger than the oldest fish on record (29 yrs, 85 cm TL), reported from the Cayman Islands (Bush et al. 2006). However, fish ≥85 cm TL have been observed in the Exuma Cays Land and Sea Park (K. Sherman pers. obs and Dahlgren unpubl. data), suggesting that individuals older than 21 yrs. may also exist in The Bahamas. A summary of population growth and mortality estimates is provided (Table 3).

Photo 3. Juvenile Nassau grouper observed amongst *Laurencia* sp.
Photo credit: Craig Dahlgren

Table 2. Summary of population growth parameters, mortality and exploitation estimates for Nassau grouper (*Epinephelus striatus*) in The Bahamas and Caribbean.

	Von Bertalanffy Growth Parameters					Mortality & Exploitation Estimates			
Location	L∞ (cm)	K	t_0	A	B	M	F	F/Z	**References**
Belize	–	–	–	0.0107	3.08	–	0.35		Carter et al. 1991; Ehrhardt and Deleveaux 2007
Cayman Islands	76.5	0.202	-0.638	–	–	–	0.21	–	Bush et al. 2006; Ehrhardt and Deleveaux 2007
Cuba	76.0 - 94.0	0.063 - 0.127	–	0.0052 - 0.1980	2.98 - 3.30	0.18	–	–	Baisre and Paez 1981; Sadovy and Eklund 1999
Jamaica	90	0.09	–	0.0107	3.11	0.17 - 0.30	–	–	Thompson and Munro 1978
Puerto Rico	–	–	–	1.26×10^{-5}	3.04	–	0.30	–	Sadovy and Eklund 1999; Ehrhardt and Deleveaux 2007
The Bahamas	–	–	–	2.14×10^{-5}	3.03	–	0.06 - 0.011	0.25 - 0.38	Sadovy and Colin 1995; Ehrhardt and Deleveaux 2007
Virgin Islands	97.4	0.185	0.488	0.0097	3.23	–	–	–	Olsen and LaPlace 1979

Status of Nassau Grouper in The Bahamas

Historically, research efforts in The Bahamas have focused on describing FSAs (e.g., Smith 1972; Colin 1997), quantifying density as part of on-going reef monitoring (Sherman et al. 2013; Dahlgren et al. 2016b; Dahlgren et al. unpubl. data), recruitment processes (Dahlgren and Eggleston 2001) or understanding ontogenetic habitat use and migratory behaviour (e.g., Sluka et al. 1996; Bolden 2000; Chiappone et al. 2000; Dahlgren et al. 2006; Dahlgren et al. 2016a). More recently, this research has been expanded to identify national research priorities outlined during the 2013 strategic planning meeting including:

1. Stock assessments of Nassau grouper FSAs
2. Perceptions of Nassau grouper
3. Catch assessments to engage fishers
4. Identification of key nursery habitats and threats to habitats
5. Movement patterns of Nassau grouper across FSAs
6. Identification of multi-species FSAs
7. Population connectivity
8. Larval dispersal and genetic connectivity
9. Geomorphology and oceanography of shelf in relation to FSA sites
10. Reproductive biology of Nassau grouper.

A summary of research activities related to these priorities is discussed below.

Density & Abundance

Quantitative fishery independent surveys of Nassau grouper populations have been conducted by divers using the Atlantic and Gulf Rapid Reef Assessment (AGRRA) protocols at over 360 reef sites over the past decade. Average density of Nassau grouper across all surveys was 0.13 fish per 100 m^2 (AGRRA: Marks and Lang 2016). The vast majority of these fish were below reproductive size, with fully protected areas such as the Exuma Cays Land and Sea park (ECLSP) having the greatest number and proportion of adult fish (>50 cm TL) surveyed (Dahlgren et al. 2014, 2016b; Dahlgren unpubl. data). However, declining trends in Nassau grouper densities for reef habitats have been documented in the ECLSP (Sherman et al. In Press).

Spawning Aggregations

Between 30-40 Nassau grouper FSAs have been reported to occur within The Bahamas. The first of these sites – Cat Cay, Bimini was described by Smith (1972), with abundance estimates ranging up to 100,000 individuals. Subsequent FSA studies were completed by Colin, Dahlgren, Eggleston and Ray (reviewed by Sherman et al. 2016), with more recent research led by Dahlgren, Sherman and Stump around Abaco, the Berry Islands, Andros, Eleuthera and Long Island (Dahlgren et al. unpubl. data; Sherman et al. unpubl. data; Sherman et al. in prep; Stump et al. 2017). Findings from current studies indicate the likely collapse of a historically active site in Andros (Stump et al. 2017) and decreased abundances at FSAs around Long Island (Dahlgren et al. unpubl. data; Sherman et al. in prep; Fig. 7). Contemporary estimates of the larger Nassau grouper FSAs within the country range in the thousands (Fig. 7). In the absence of baseline data for most FSAs (Fig. 7) and known variability in fish biomass during the spawning period (Dahlgren et al. 2016a; Sherman et al. in prep) continued monitoring will be required to provide reliable estimates of spawning stock biomass for

other reported FSAs. Monitoring data will be important to assess the effectiveness of management practices and refine policies for the conservation of the species.

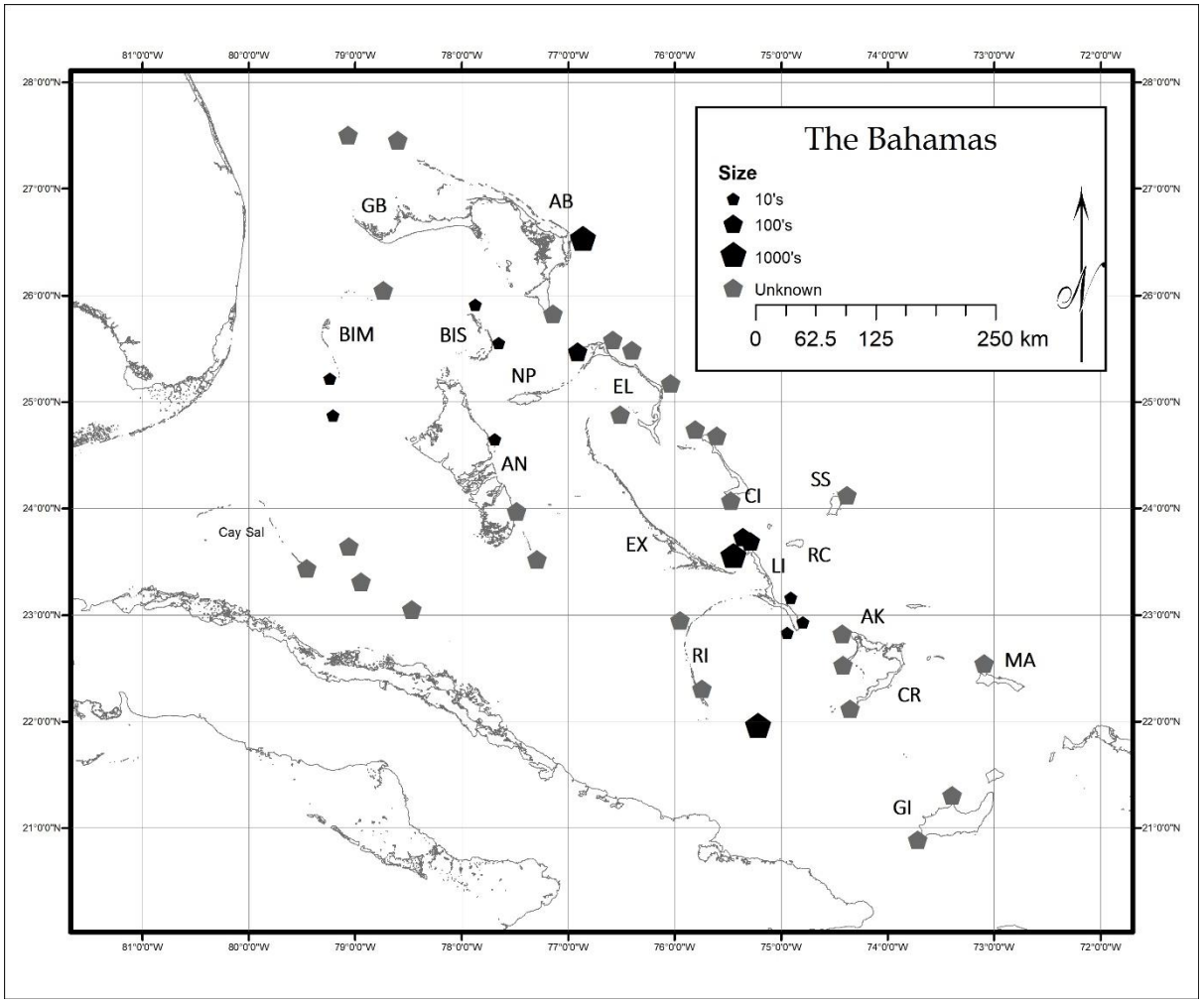


Figure 7. Status of Nassau groper FSAs in The Bahamas as of December 2017.
Map: *Lindy Knowles*

Geomorphology & Oceanography

Efforts to investigate the geomorphology and oceanography of the shelf in relation to FSAs have been limited to bathymetric mapping (i.e. mapping the topography and depths of the ocean floor) and the deployment of drifters (used to track sub-surface currents). Over the past three years, we have mapped and characterized benthic habitats and topographic features around five reported Nassau grouper spawning aggregations: High Cay (Fig. 8), Hail Mary (Fig. 9), North Point (Fig. 10), Newton's Cay (Fig. 11), and Little Egg Island (Fig. 12).

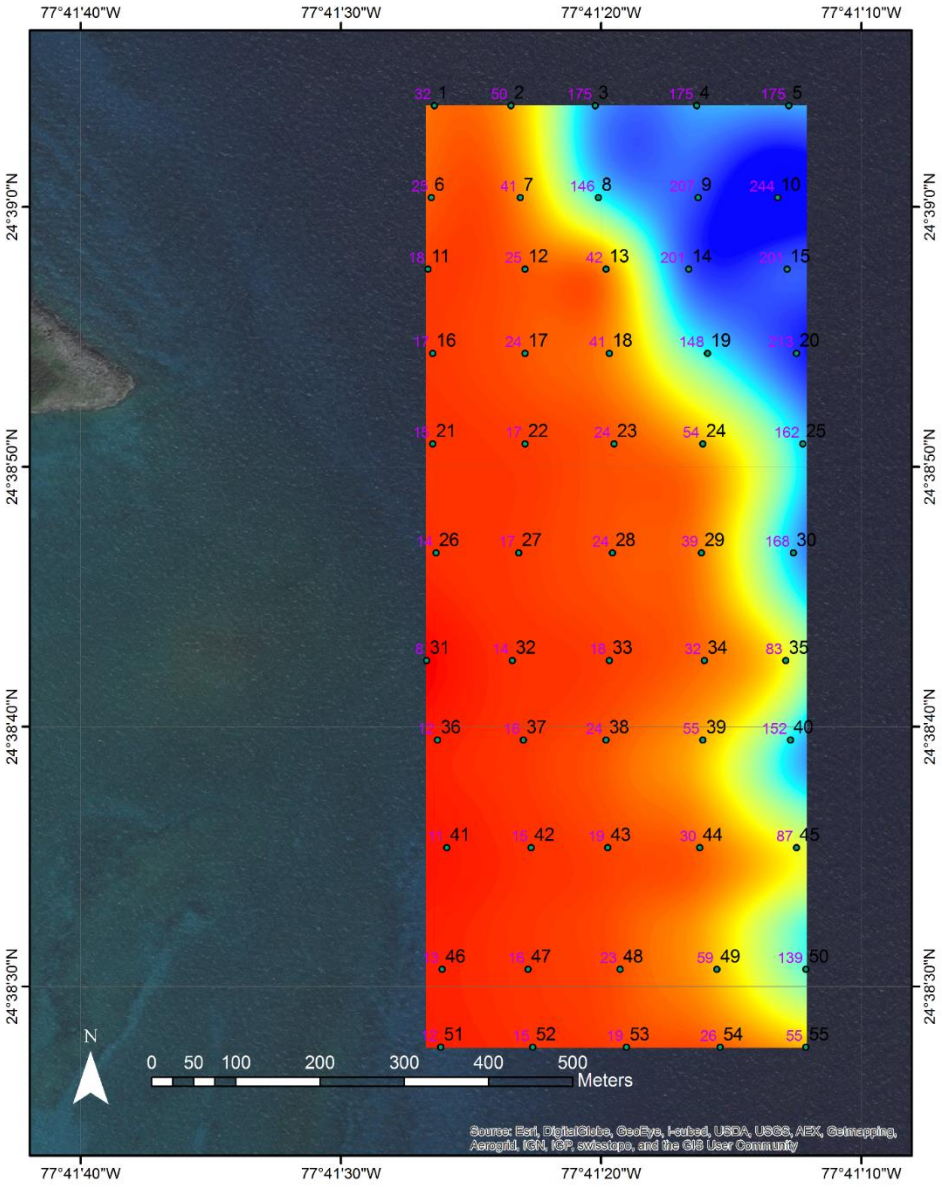


Figure 8. Bathymetry map of the High Cay FSA.
Map: *Gwilym Rolands* (Living Oceans Foundation).

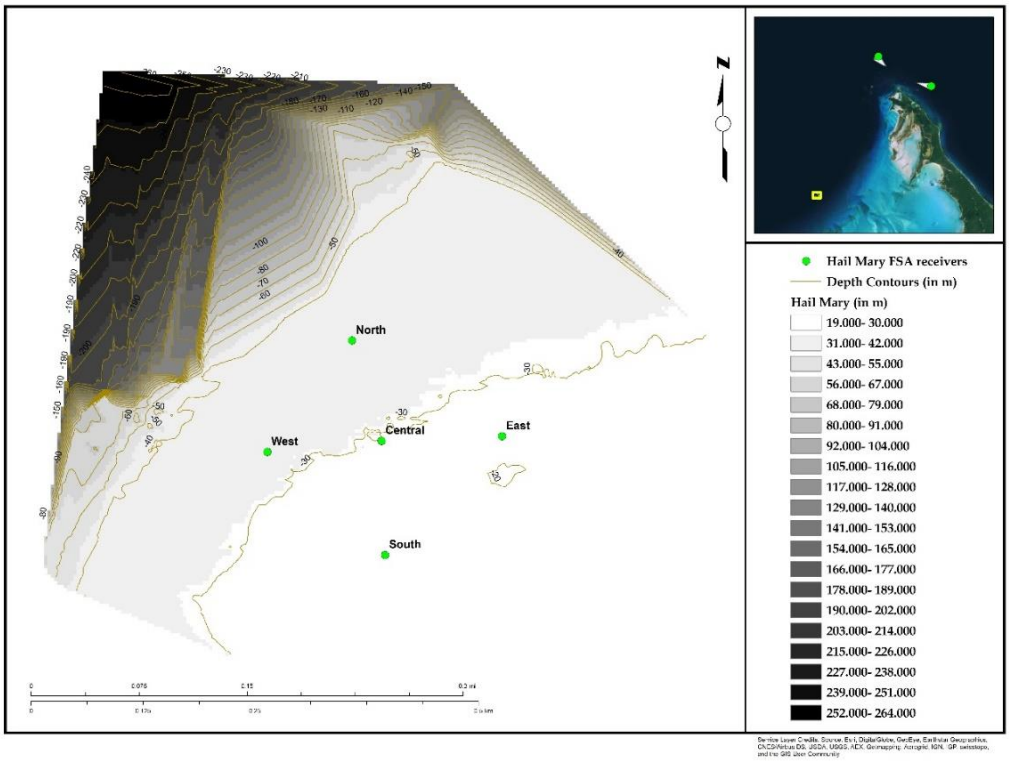


Figure 9. Bathymetry map of the Hail Mary FSA.
Map: *Lindy Knowles*

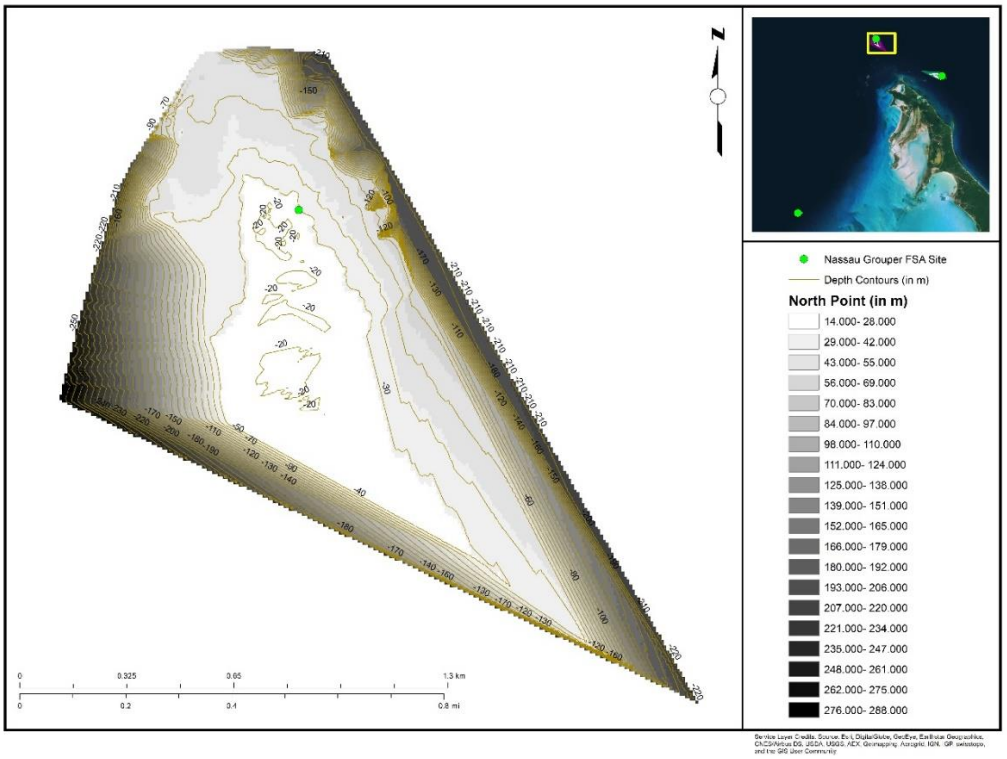


Figure 10. Bathymetry map of the North Point FSA.
Map: *Lindy Knowles*

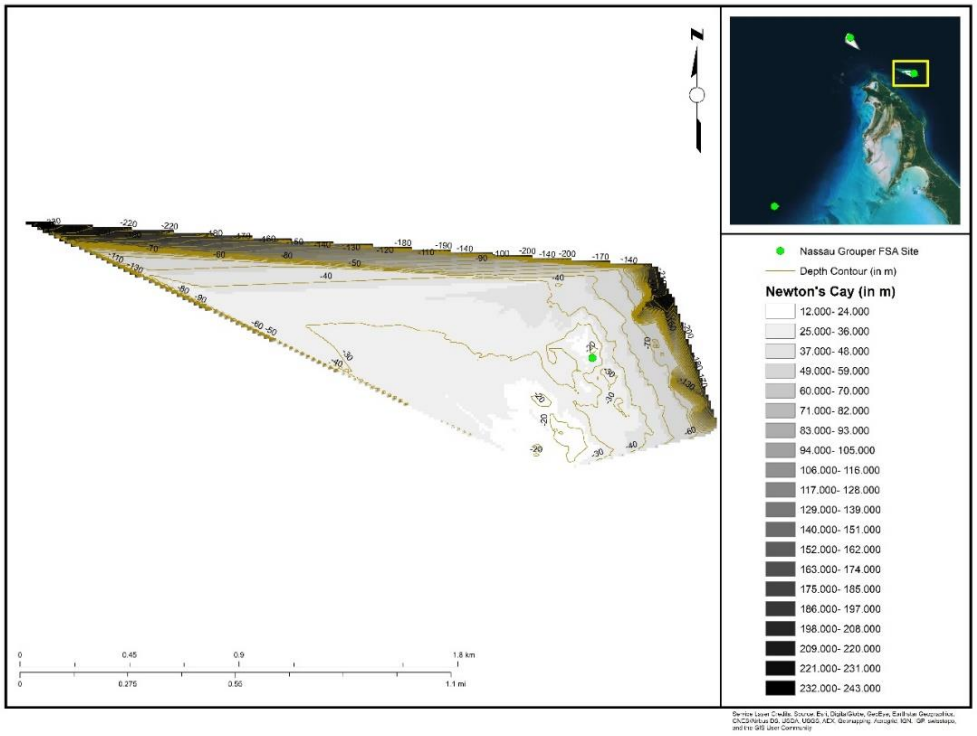


Figure 11. Bathymetry map of the Newton's Cay FSA.
Map: *Lindy Knowles*

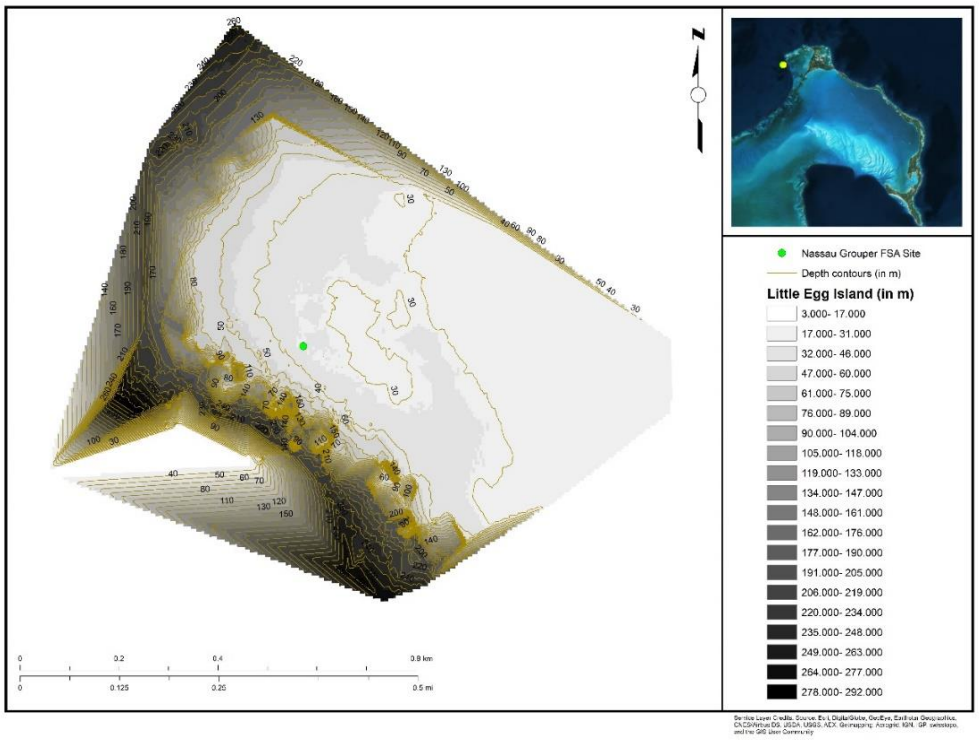


Figure 12. Bathymetry map of the Little Egg Island FSA.
Map: *Lindy Knowles*

Oceanographic patterns have been explored in preliminary work using drifters deployed around two Nassau grouper FSAs. Track data from drifters released at the Hopetown FSA in January 2016 showed that surface currents carried drifters out into the Atlantic Ocean. However, after 30 days, they looped back to the northwest portion of the Little Bahama Bank, crossing reef habitat around the time larval Nassau grouper would be expected to recruit (i.e. settle) into nursery habitats. Conversely, drifters released from the Hail Mary FSA revealed northwest moving currents, with tracks following along the edge of the Exuma Sound. Future research is planned to develop a larval biophysical model for Nassau grouper, which integrates genetic data, larval behaviour and oceanographic conditions (Paris, Sherman and Dahlgren) to better understand source-sink dynamics and the main driver(s) of connectivity.

Movement Patterns

To date several studies have examined movement of Nassau grouper through their development, within home ranges and during spawning migrations. Studies by Dahlgren and Eggleston documented habitat use by juvenile Nassau grouper and tracked ontogenetic habitat shifts as they grow and develop, from settling into off-reef habitats such as clumps of the seaweed *Laurencia* spp. in tidal creeks and sounds to small patch reefs in seagrass beds during the first year of their life before moving to larger reef systems, often several kilometers away (Eggleston 1995, Dahlgren and Eggleston 2000; 2001). For older subadult and adult Nassau grouper, research of tagged fish (i.e., visual mark recapture/re-sightings and acoustic telemetry studies) show a small home range size. For example, a study of visually and acoustically tagged fish on patch reefs ranging in size from 15-35 cm showed little movement between reefs spaces ~250 m apart and movement rarely exceeding 10 m off the home reef (Dahlgren, unpublished data). Adult Nassau grouper in the ECLSP, similarly showed a home range of only 18,305 m^2 on average (Bolden 2001). In contrast to these small home range sizes, adult Nassau grouper larger than 50 cm TL tagged with acoustic transmitters and/or externally visible (e.g. Floy™) tags have been shown to make long distance spawning migrations of 35 to over 200 km along the shelf edge in The Bahamas (e.g., Bolden 2000, Dahlgren et al. 2016a, Stump et al. 2017). These migrations typically last up to 1-2 weeks with fish swimming at relatively constant speeds averaging 1.3 to 1.7 km/hr to and from spawning sites where they stay to spawn for only 1-2 days (Dahlgren et al. 2016a; Stump et al. 2017) before returning to their home reef. In some cases, however, fish may spend a month or more away from their home range during spawning times when the first full moon of the spawning season is early in the season (i.e., late November or first week of December) and water temperatures remain warm (Dahlgren et al. 2016a).

Populations Genetics & Connectivity

Molecular markers commonly used for investigations of genetic connectivity and population structure include microsatellites and single nucleotide polymorphisms (SNPs). Recently, both markers have been employed to assess the genetic health of Nassau grouper within The Bahamas. Microsatellite analysis has shown that Nassau grouper have undergone drastic declines in their effective population size (N_e) and contemporary values of allelic richness (an indicator of genetic diversity) are low compared to other species (Sherman et al. 2017). However, estimates of genetic differentiation were low and values of heterozygosity were similar across locations, implying moderate to high levels of gene flow within the country (Sherman et al. 2017). Moreover, two genetic stocks of Nassau grouper appear to exist in The Bahamas, but one stock is less abundant (Fig. 13; Sherman et al. 2017). Indeed, if there are genuinely two genetic stocks (i.e. population

structure), they do not appear to be driven by contemporary geography or geographical barriers to gene flow. Instead, this pattern may be reflective of either the historical background of both stocks, and/or some other intrinsic (non-geographic) factor. Thus, these findings further underscore the need for better management of the species.

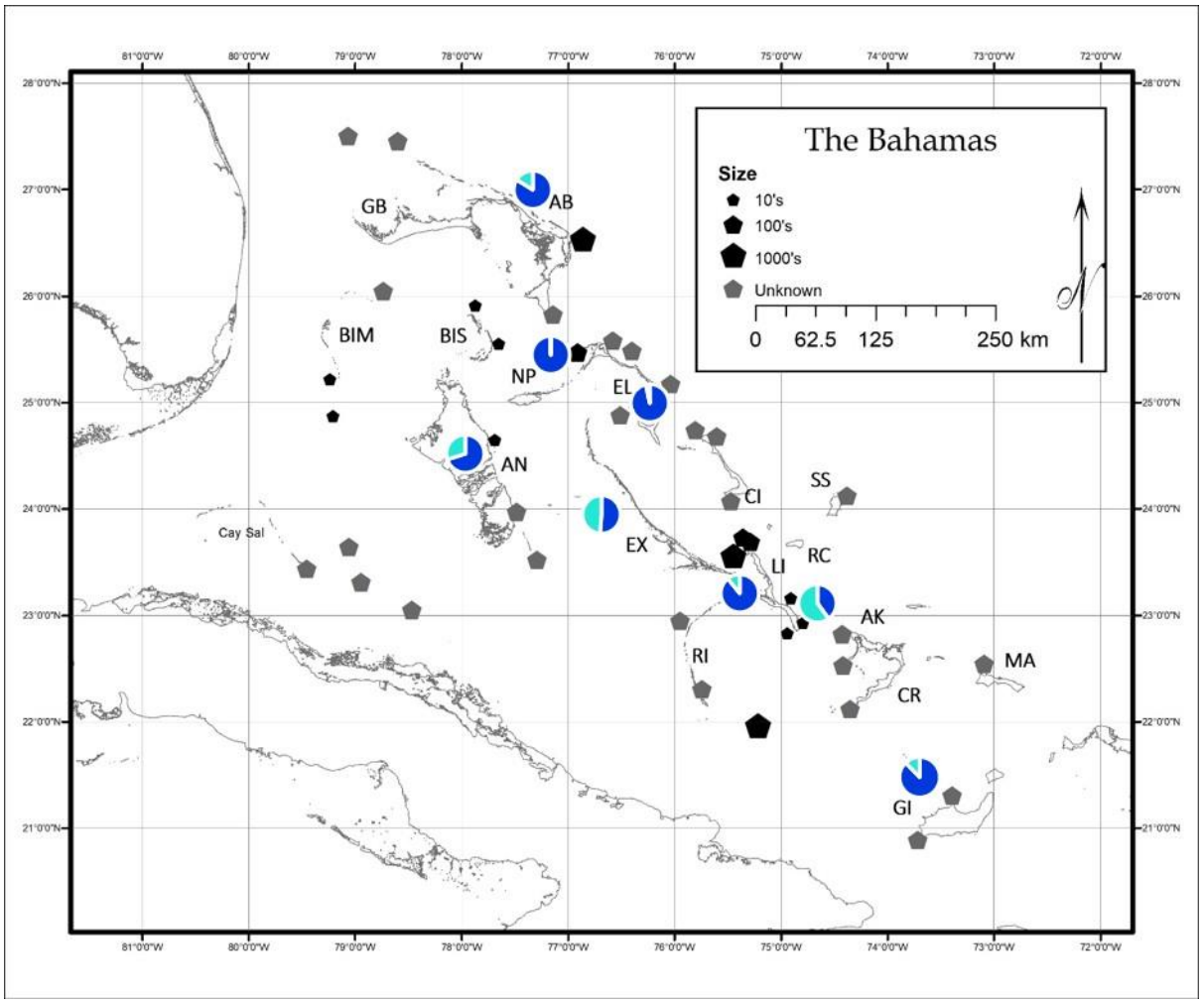


Figure 13. Genetic composition depicting the two stocks of Nassau grouper within The Bahamas overlaid on FSA map. Based on microsatellite data from Sherman et al. (2017).

Emerging results from SNP data are providing insight into patterns of gene flow (i.e. genetic connectivity) and evidence in support of population structure that was not observed through microsatellite analysis (Sherman el al. 2017; Sherman et al. in prep). Future research is being planned to integrate biophysical modelling with population genetics to provide additional insights into the mechanisms influencing source-sink dynamics for the species (Paris, Sherman and Dahlgren). These findings will have direct implications for improving Nassau grouper management within The Bahamas.

Stakeholder Assessments

Under the theme "Reversing the Decline", a Nassau Grouper Fishery Management Workshop was held on March 17th, 2016 during the third Bahamas Natural History Conference to

outline key components and promote the establishment of a comprehensive national Nassau grouper sustainable management plan for The Bahamas (Appendix D). The workshop consisted of 15 stakeholders including policy-makers, law enforcement officials, fishermen, marine resource managers, scientists, non-governmental organizations (NGOs) and the private sector. Participants completed a SWOT analysis, identifying 10 strengths, 18 weaknesses, 17 opportunities and 11 threats of the Nassau grouper fishery (Table 3).

Table 3. SWOT analysis of the Bahamian Nassau grouper fishery. Results are reported verbatim.

Strengths	Weaknesses	Opportunities	Threats
We know that the species is threatened	Schooling behaviour for spawning makes them vulnerable	Need fishery-independent data to help management	Overfishing/Probably already below critical population threshold
Fixed closed season	Decreasing number/distribution of spawning schools	Data collection has improved	Low spawning stock biomass
Viable population in The Bahamas	Unsustainable to fish spawning aggregations that are well known by fishermen and easy to harvest	New Fisheries Officers could enforce current regulations	Lack of compliance (e.g. illegal fishing during the closed season by foreigners and Bahamians and catching undersized fish)
Economic benefits (direct & indirect, e.g. > $1 mil in revenue, trickle-down economics, higher valued compared to other fish species, etc.)	Need to amend closed season to include November & March due to early and late spawning	RBDF and Police could better support enforcement and research	Lack of enforcement
Food source/diet (grouper bigger than other fish)	Poor enforcement	Training for Fisheries Officers (e.g. why regulations exist, what they are, conflict resolution, etc.)	High demand by Bahamians and foreigners
Compliance by many Bahamians for closed season	Family/community connection decreases willingness to enforce regulations	Increase fines for Bahamians and foreigners violating regulations	No gear restrictions (e.g. hookah rigs, no trap limits)
Public understands importance of species	Lack of education of fishermen	Change policy to allow ticketing/administrative fines	No bag limits/quotas
Science/research is on-going	Lack of knowledge of regulations by public	Communication strategy for changes to Fisheries Regulation 35	Lack of knowledge/Misconceptions about biology/behaviour (e.g. Illegal fishing creates a "catch it while you can" mentality. Tragedy of the Commons)
Cultural value/benefits	Boom fishery	Fishermen willing to participate in collaborative patrolling/enforcement	Lack of alternative/sustainable livelihood options for fishermen
More fisheries officers recently added to DMR	Monitoring of fishery	Some fishermen keen to get involved in research	Lionfish
	Lack of funding for scientific research and FSA monitoring	Explore sustainable harvesting strategies for other species	Potential climate change impacts (e.g. OA data deficient)

	Source-sink dynamics unknown Not dealing with markets buying grouper but rather targeting fishermen Educational/outreach materials not targeting right age groups Lack of support for fishermen (e.g. tax/duty free breaks) Bureaucratic decision-making processes/Lack of governmental mechanisms to allow willing fishermen to participate in enforcement Need N. Grouper Fisheries Improvement Plan Working Group Lack of sustainable fisheries plan for grouper	Intervene in markets (supply-demand chain)/outreach campaign designed for restaurants, hotels, fish processing plants, tourists, etc. More public awareness programs (e.g. local meetings, fishermen focus groups, workshops, walk-a-bouts, etc.) Develop material for recreational fishing and diving tourists Conserve pelagic environment important for larvae Collaboration among conservation partners to support development of sustainable fishery management plan Aquaculture potential	

To further explore and assess stakeholder perspectives regarding the status and management of the fishery, a questionnaire was developed incorporating aspects of the SWOT analysis and distributed to relevant individuals. Key results (from Sherman and Tyler. in prep) are summarized below:

1.) Conservation and enforcement stakeholders have varied opinions regarding the current state of the fishery and how it should be managed.
2.) Both stakeholder groups are in favour of amending existing regulations and in support of new regulations.
3.) Most enforcement officers believe existing penalties for violating fishery regulations should increase.
4.) Enforcement stakeholders have conflicting opinions about which enforcement methods are the most effective for managing the fishery.
5.) Conservation and enforcement stakeholders do not view current education and outreach efforts as very effective.
6.) Stakeholders are supportive of quick action to strengthen protection for Nassau grouper.

Importance of a National Conservation Management Plan for Nassau Grouper

Biological Justification for Conservation

Predator-prey interactions are important for shaping and maintaining trophic structure in both marine and terrestrial ecosystems. Nassau grouper are important top predators of coral reef habitats and are also a food source for larger apex predators, e.g. sharks (Eggleston et al. 1998; Stallings 2008; Mumby et al. 2012; O'Farrell et al. 2014). Moreover, their formation of annual FSAs is not only responsible for replenishing fish stocks, but also for the addition of key nutrients (e.g., nitrogen and phosphorous) that are important for coral reef health (Archer et al. 2015). The protection of FSAs has been identified as a biodiversity target for The Bahamas (Moultrie 2012) and has been incorporated into Marxan analyses for the establishment of new MPAs (Moultrie & Moss-Hackett 2014).

Socioeconomic & Cultural Value

Nassau grouper are a highly prized commercial and recreational fish species (Buchan 2000; Cushion and Sullivan-Sealey 2008) and are also an integral part of Bahamian culture. Commercial landings data from the Department of Marine Resources (DMR) indicate that commercial harvest peaked at 514 tonnes in 1997, but have declined by 86% over the past twenty years (Sherman et al. in 2016). The value of commercially landed Nassau grouper has also declined from nearly $3 million to less than $1 million (Sherman et al. 2016).

Threats to Nassau Grouper

Fishery pressure

High market demand ($120-200 per Nassau grouper) coupled with the illegal capture of sexually immature fish, legal capture of sexually immature and/or mature but inexperienced spawners (i.e. fish ≤48 cm TL that may or may not have undertaken first spawning migration), and illegal capture of fish from FSAs all impede population recovery. Individuals are typically removed from the fishery between 2-9 years old (Bush et al. 1996). The perception of fishers that spawning sites are still supporting large numbers of fish is often inaccurate due to hyperstability (Sadovy de Mitcheson and Erisman 2012; Figure 14). Moreover, no quotas or bag limits have been established (for Bahamian commercial fishers) and fishing gears for catching Nassau grouper are unrestricted with the exception spearguns and fishing on SCUBA, which are prohibited in the country. The bag limit for foreign recreational fishers is 20 lbs of reef fish (not exceeding 60 lbs maximum in total weight) and 250 lbs per vessel is restricted for non-commercial Bahamian fishers.

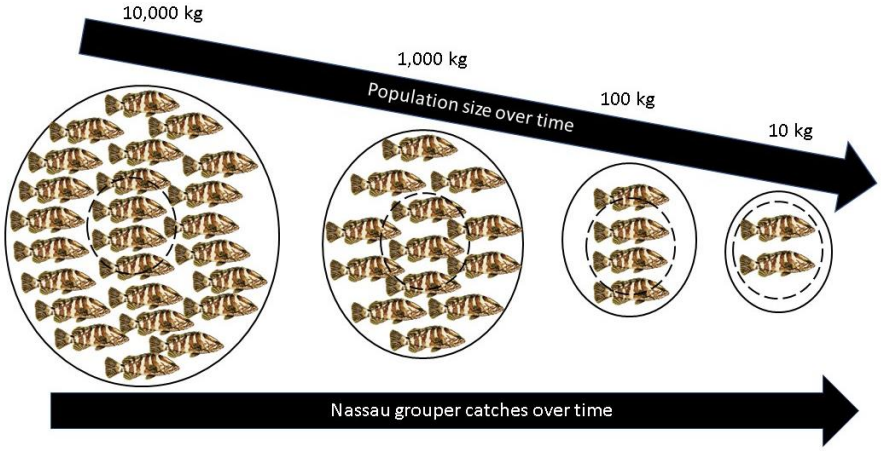


Figure 14. Consequences of spawning aggregation fishing. Recreated based on image from Sadovy de Mitcheson and Erisman 2012.

Threat: Overfishing

Actions: 1) Eliminate fishery pressure from foreign and Bahamian fishers at Nassau grouper FSAs

2) Enforce existing policies and amend fishery regulations to reduce fishing pressure and promote sustainable harvesting.

3) Remove **all** grouper species from the sportsfishing permit

4) Strengthen regional and international relationships to deter illegal fishing by foreigners (i.e. "poaching").

Habitat Degradation & Loss

Nassau grouper utilize a variety of habitats throughout their life cycle. However, the habitats which are most impacted include mangroves and coral reefs. Mangroves and other nursery habitats (e.g. seagrass beds) are often severely impacted by coastal development and associated pollution (Lotze et al. 2006). Coral reefs are being impacted by a range of anthropogenic activities including unsustainable fishing, invasive species, increasing incidences of coral bleaching and disease (Gardner et al. 2003; Allvarez-Filip et al. 2015). Degradation and loss of these essential habitats will impact both juvenile and adult fish.

Threat: Habitat degradation and loss

Actions: 1) Stringent monitoring of coastal development to minimize pollution and damage to key habitats

2) Protection of critical Nassau grouper habitats through MPA and marine reserve designation.

3) Support habitat restoration programmes (e.g., coral reef and mangrove restoration).

Invasive Alien Species

Several marine fish species have been introduced into the Atlantic Ocean (Schofield et al. 2009), including invasive species (de Castro et al. 2017), and have the potential to impact native reef fish composition (Albins and Hixon 2008). For example, invasive Indo-pacific lionfishes (*Pterois volitans/miles*), established within The Bahamas since 2004 (Schofield 2010), opportunistically consume Nassau grouper (Morris and Akins 2009; Muñoz et al. 2011; Layman and Allgeier 2012) and compete with them for both food and space (O'Farrell et al. 2014; Raymond et al. 2015). High densities (~393 lionfish ha^{-1}) of invasive lionfishes in The Bahamas (Green and Côté 2009) significantly affect recruitment processes of native fish species (Albins and Hixon 2008) and can retard recovery of an already declining Nassau grouper population through resource competition and predation.

Threat: Invasive species (e.g., lionfish)

Action: 1) Continued native reef fish monitoring (see Monitoring Plan for BNPAS)

2) Lionfish removal in critical marine habitats (see National Lionfish Response Plan; CSA; NISS; Smith et al. 2017)

3) Implementation of prevention and control strategies outlined in the NISS.

We also recommend shifting the timing of lionfish tournaments to December or another month during the Nassau grouper closed season to offer an alternative source of revenue to fishers, which would also assist with on-going lionfish removal efforts.

Disease & Predation

Incidences of disease and infections in Nassau grouper are rare. However, parasitic tapeworms, nematodes and isopods have been documented in the eyes, nostrils, bucaal cavity, gills and stomach of fish collected from Florida, Jamaica and the Cayman Islands (Thompson and Munro 1978; Sadovy and Eklund 1999; Semmens et al. 2006. Additionally, parasitic isopods (e.g. *Excorallana tricornis tricornis*) have been shown to infest fish following spawning events in the Cayman Islands (Semmens et al. 2006). This is likely to increase their susceptibility to capture and predation. However, with low natural morality rates and few documented natural predators including great barracuda (*Sphyraena barracuda*), carnivorous sharks, and conspecifics (Albins et al. 2009), fishery pressure is still the biggest threat affecting recovery of Nassau grouper populations.

Threats: Parasites and predators

Action: 1) Eliminate fishery pressure at Nassau grouper FSAs.

Climate Change

Hypothesized ecosystem responses to climate change include poleward migration and redistribution of organisms, population collapses, local extinctions, disruptions to large-scale migrations, and alterations to food availability and trophic structure (Pörtner and Farrell 2008). Fish exhibit a range of responses to climate-induced stressors (Munday et al. 2009; Crozier and Hutchings 2014). Temperature changes are known to affect larval development (Ellis et al. 1997) and influence spawning for Nassau grouper (Colin 1992). However, predictions on how Nassau grouper behaviour and distribution could change as ocean temperatures and acidity increase due to climate change are

data deficient. Coral reefs are being affected by thermal stress (Hughes et al. 2003; Hoegh-Guldberg et al. 2007) and a reduction or loss of reef habitat is likely to negatively impact Nassau grouper. A delayed start to the spawning season has also been predicted (Asch and Erisman unpubl. data). While climate change does pose a threat to both Nassau grouper and their habitats, unustainable fishing practices represent a more immediate threat to the species.

Threat: Climate Change

Actions: 1) Assess impacts of climate change-linked abiotic factors on Nassau grouper

2) Support monitoring and restoration programmes for critical habitats (e.g. coral reefs and mangroves) to promote resilient ecosystems (see Monitoring Plan for BNPAS; Reverse The Decline Project).

Table 4. Threat Assessment for Nassau grouper (*Epinephelus striatus*). Threats were assigned a score for severity (low = 1, medium = 2, high = 3) and restoration likelihood (unlikely = 1, possible = 2, likely = 3) then multiplied to obtain an overall threat rating score. For the overall threat rating, scores ranging between 1-3 = low, 4-6 = medium, and 7-9 = high. Higher scores (in red) indicate priority areas of concern, which can be mitigated with appropriate management.

Threats	Limits to Species Recovery	Source of Threats	Threat Severity (Score A)	Restoration Likelihood (Score B)	Total Threat Score (A x B)	Overall Threat Rating
Fishery Pressure						
Nassau grouper FSA fishing (during closed season)	Removes large quantities of spawning fish biomass; likely to reduce diversity and negatively impact reproductive success of FSAs	Commercial, recreational & subsistence fishing on FSAs	3	3	9	High
Legal removal of sexually immature or inexperienced spawners (i.e. 3 lb. fish)	Likely to delay population recovery because fish are not spawning before removal for the fishery	Commercial, recreational & subsistence fisheries	3	3	9	High
Unrestricted fishing gears	Use of hookahs and compressors, fish traps/pots, at or around FSAs increases potential to remove large quantities of fish biomass compared to other fishing gears (e.g. hand-line or rod-and-reel)	Commercial, recreational & subsistence fisheries	3	3	9	High
Ghost traps at FSAs	Mortality of fish (adults & juveniles)	Commercial, recreational & subsistence fisheries	3	2	6	Medium
IUU fishing (during open season)	Removes unknown quantities of fish biomass	Illegal capture of fish by Bahamians and foreigners	3	1	3	Low

Habitat Degradation & Loss						
Degradation/Loss of mangroves	Limits available habitat and food for recruits and juveniles; increases predation risk; likely to reduce long-term survival of recruits and juveniles	Mangrove destruction for coastal development	3	3	9	High
Degradation/loss of coral reefs	Limits available habitat and food for sub-adults and adults; increases predation risk; likely to reduce long-term survival of sub-adults and adults	Coral bleaching, coral diseases, loss of grazers (i.e. urchins, parrotfish, etc.)	3	2	6	Medium
Reduced larval development/survival due to poor water quality	Reduces number of recruits	Eutrophication, effluent discharges, oil spills, etc.	2	2	4	Medium
Compromised fish health	Impaired development/reproductive output/feeding	Eutrophication, effluent discharges, oil spills, etc.	2	2	4	Medium
Loss of seagrass	Limits available habitat and food for recruits and juveniles; increases predation risk; likely to reduce long-term survival of recruits and juveniles	Smothering or removal of seagrass for coastal development	3	1	3	Low
Invasive Alien Species						
Lionfish predation and competition	Removal of juveniles; decreases available Nassau grouper prey	High densities of invasive lionfish	1	2	2	Low
Disease & Predation						
Diseases & parasites	Likely to lead to increased incidences of infections or mortality of fish	Parasitic isopods, trematodes, etc.	1	1	1	Low

Natural predators	Mortality of fish	Sharks, Great barracuda, Nassau grouper	1	1	1	Low
Climate Change						
Forecasted increases in SSTs	Possible reduced larval development/survival	Anthropogenic-induced climate change	2	1	2	Low
	Possible shifts in spawning occurrence	Anthropogenic-induced climate change	2	1	2	Low
Ocean acidification	Unknown	Anthropogenic-induced climate change	1	1	1	Low

Nassau Grouper Conservation & Management

National Policies and Regulations

The legislative framework governing the management of the Nassau grouper fishery in The Bahamas is the Fisheries Resources Jurisdiction and Conservation Act. Fisheries regulations are created under the umbrella of this Act via the Fisheries Resources (Jurisdiction and Conservation) Regulations (http://laws.bahamas.gov.bs/cms/en/). Information regarding protected species, gear usage, quotas, size limits, closed seasons, fish processing, fishing permits and licenses, and penalties for offenses are outlined within the regulations, which were established to conserve and manage fishery resources for the benefit of Bahamians. By law, only Bahamians or citizens of The Bahamas that legally reside within the country are permitted to engage in commercial fishing activities. A permit is required for any foreign vessel engaged in recreational or sportsfishing within the EEZ of The Bahamas. A prerequisite for the permit is clearance at an official port of entry by Customs officials or the Department of Marine Resources (DMR). A maximum of six rods or reels is usually permitted along with the catch of up to 20 lbs of reef fish (not exceeding 60 lbs maximum in total weight). The use of spearguns is illegal and commercial fishing on SCUBA is not allowed. The Fisheries Act (Regulation 35 of Sub. Leg. Vol. IV, Ch. 244-3) was amended on September 29th, 2015 and current regulations pertinent to Nassau grouper are outlined as follows:

- Landed Nassau grouper must be ≥3 lb.
- "During the closed season, no person shall land any fish commonly known as "grouper" unless its head, tail and skin is intact.
- No person shall take, land, process, sell or offer for sale any fish commonly known as "Nassau grouper" during the closed season, except where such taking or landing is carried out with the written approval of the Director of Fisheries for scientific research purposes.
- For the purposes of Regulation 35, "closed season" means the period commencing on the 1st day of December in any year and ending on the 28th of February of the immediate succeeding year".

International Policies, Regulations, Agreements & Conventions

Wold Conservation for Nature (IUCN) Red List

Despite more than 30 years of protective measures, Nassau grouper populations in most countries have substantially decreased. Rapid global declines (≥ 60%) in Nassau grouper populations coupled with the extirpation of historic FSAs and continued fishery pressure led to its most recent listing as critically endangered by the IUCN (re-assessed by Carpenter et al. 2015). Critically endangered species on the Red List v. 3.1 are defined as species "facing an **extremely high risk** of extinction in the wild" (http://www.iucnredlist.org/static/categories_criteria_3_1).

Endangered Species Act (ESA)

The United States Endangered Species Act (ESA) was established in 1973 to prevent the extinction of threatened and endangered species. Under this act, threatened species are classified as those which are "likely to become an endangered species within the foreseeable future throughout all or a significant portion of its range" (ESA 1973). Following a petition initiated by WildEarth

Guardians in 2010, Nassau grouper was officially listed on the ESA (81 FR 42268) in June 2016 as a threatened species (https://www.federalregister.gov/articles/2016/06/29/2016-15101/endangered-and-threatened-wildlife-and-plants-final-listing-determination-on-the-proposal-to-list). Stipulations of this act mean that a US federal recovery plan will be created to restore Nassau grouper populations and if successfully implemented, should eventually result in its downlisting.

Convention on Biological Diversity (CBD)

Conserving biodiversity is the primary objective of the Convention of Biological Diversity (CBD). After ratification in 1993, The Bahamas pledged to adhere to principles stipulated under the CBD's Articles. Several obligations relevant to the protection of Nassau grouper and its associated habitats are highlighted:

- "Develop national strategies, plans or programmes for the conservation and sustainable use of biodiversity or adapt for this purpose existing strategies, plans or programmes (Article 6).
- Identify and monitor components of biodiversity that need to be conserved and used sustainably (Article 7).
- Promote the protection of ecosystems, natural habitats and the maintenance of viable populations of species in natural surroundings (Article 8).
- Develop or maintain necessary legislation and/or other regulatory provisions for the protection of threatened species and populations (Article 8)
- Rehabilitate and restore degraded ecosystems and promote the recovery of threatened species (Article 8).
- Promote and encourage research which contributes to the conservation and sustainable use of biodiversity (Article 12)".

Progress in achieving these objectives is overseen by the Bahamas Environment Science and Technology (BEST) Commission with the support of National Implementation Support Programme (NISP) partner agencies, which also include the BNT, DMR and The Nature Conservancy (TNC).

Specially Protected Areas and Wildlife (SPAW) Protocol

The SPAW protocol under the Cartagena Convention was designed to support existing international conservation and environmental sustainability efforts (e.g. CBD). The Bahamas ratified the SPAW protocol in 2010. One of the objectives of the protocol is to "support the conservation of threatened and endangered species and sustainable use of natural resources to prevent them from being threatened or endangered". Most of the work under the SPAW protocol has been directed toward sea turtles, invasive species and marine mammals. However, Nassau grouper was recently listed under the SPAW protocol (SPAW annex III), and as a critically endangered species, the development of a species-specific national plan for Nassau grouper would represent an important contribution to ongoing conservation management efforts in The Bahamas.

The Lacey Act

The Lacey Act is a federal law in the United States of America that was enacted to protect wildlife. "Under the Lacey Act**,** it is unlawful to import, export, sell, acquire, or purchase fish, wildlife or plants that are taken, possessed, transported, or sold: 1) in violation of U.S. or Indian law, or 2) in interstate or foreign commerce involving any fish, wildlife, or plants taken possessed or sold in violation of State or foreign law". As such, the Lacey Act provides a measure of deterring IUU fishing by U.S. citizens.

Regional Policies and Regulations

As a member of the Caribbean Regional Fisheries Mechanism (CRFM), The Bahamas is collaborating with 16 (English speaking) Caribbean countries to tackle issues related to the sustainable harvest and management of fisheries resources (CRFM 2013). The Bahamas has also been involved with regional meetings of the CRFM, Western Central Atlantic Fishery Commission (WECAFC) and Caribbean Fisheries Management Council (CFMC) working group for spawning aggregations. This working group successfully advocated for Nassau grouper to be added to the SPAW protocol. Strengthened national and regional support to achieve sustainable management and other outlined CRFM strategic objectives should complement on-going national management efforts for the Bahamian Nassau grouper fishery. Spanish speaking countries such as Cuba and the Dominican Republic are not formally part of the CRFM. Bilateral discussions with the Dominican Republic have occurred to address IUU fishing via a Technical Cooperation Agreement, but were unsuccessful. Continued efforts to address IUU fishing with Spanish speaking countries are encouraged.

National Management Authorities

The following governmental and non-governmental entities are **legally** tasked with the management of Nassau grouper (i.e. through enforcement or habitat protection) in The Bahamas:

1. Department of Marine Resources
2. Royal Bahamas Defence Force
3. Royal Bahamas Police Force
4. Bahamas National Trust
5. Customs.

Recommendations for Advancing Conservation & Management

We have reviewed and assessed the adequacy of existing regulatory mechanisms related to the management of Nassau grouper in The Bahamas. Based on our evaluation, two existing regulations should be revised as they fail to assist with population recovery and by extension a sustainable fishery. In addition, we also recommend the establishment and implementation of new regulations. Justifications for these amendments are also provided.

Revisions to Existing Regulations

Regulation: **Amendment #1 Extend closed season (1 November-March 31)**

Justification for Amendment: Telemetry data and visual observations indicate migrations to FSAs also occur during November and March if the timing of the full moon falls either early or late in the month. Moreover, emerging research has predicted shifts in the timing of spawning due to increased sea surface temperatures (Asch and Erisman unpubl. data). Amending the existing regulation to protect early and late spawners would prevent the (currently) legal capture of spawning individuals during the reproductive season. We recommend changing the wording of the closed season to prohibit the fishing, sale and possession of Nassau grouper **during the entire spawning period**.

Regulation: **Amendment #2 Increase size limit to ≥54 cm (21.3 in) TL / ~4 kg (9 lb)**

Justification for Amendment: Approximately 77% of fish are ≥ 54 cm TL when they first migrate to spawn. The average weight of tagged fish ≥ 54 cm TL was ~4 kg or 9 lb (Fig. 15). The current 3 lb

regulation results in the removal of Nassau grouper from the fishery **before** they contribute to stock replenishment (Fig. 15). Moreover, fishers and consumers purchasing Nassau grouper are unlikely to know exactly how big a 3 lb fish is, which may lead to the continued capture of undersized fish (e.g. Photo 4). We strongly recommend amending the fishery regulation to **ban harvesting any Nassau grouper <54 cm TL (21.3 in)**. Additionally, we suggest the inclusion of both metrics of size (i.e. length and weight) in the fishery regulation and educational materials moving forward. As a supportive measure, DMR is advised to advocate for a complete ban of selling and serving any Nassau grouper (fresh or frozen) during the seasonal closure.

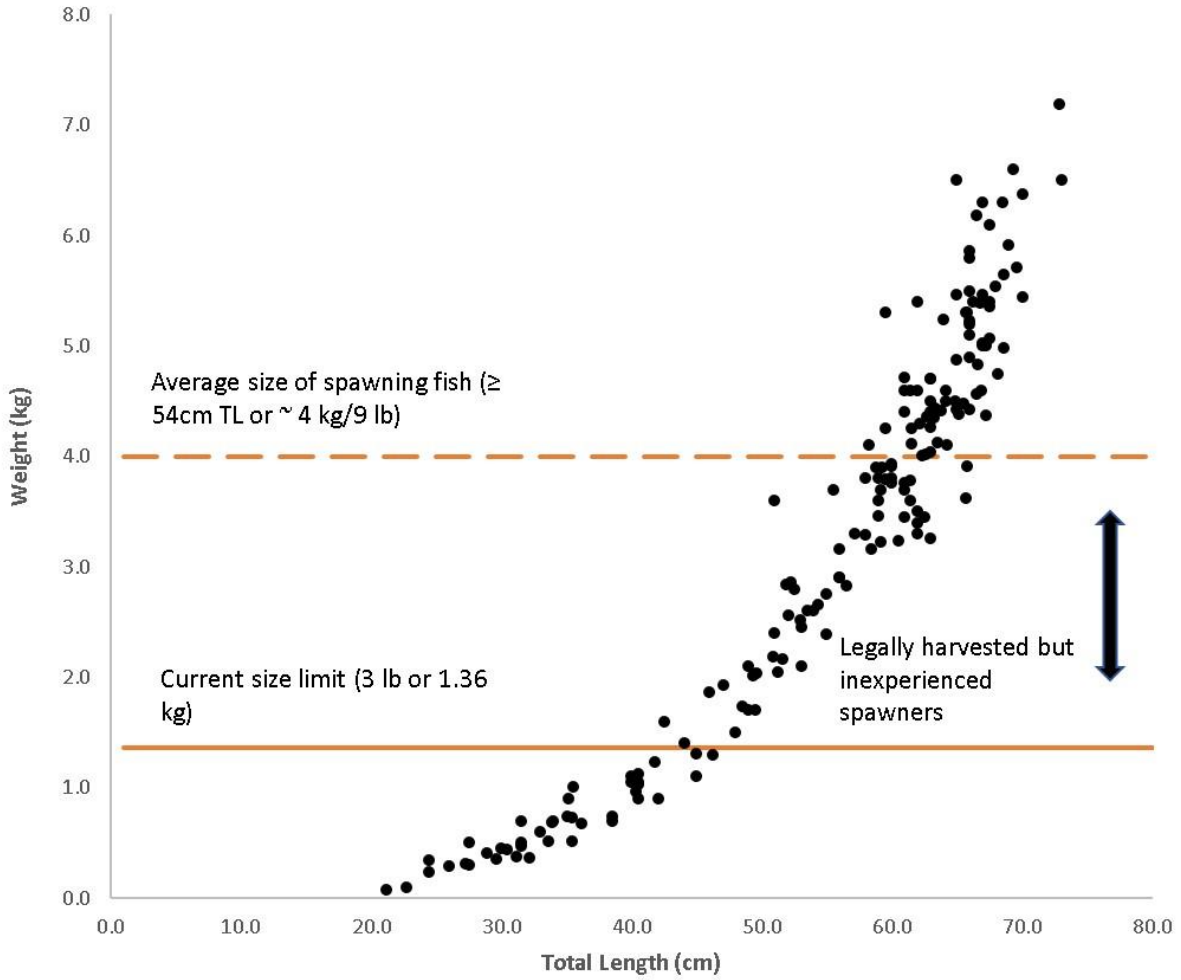


Figure 15. Morphometric data from externally and surgically tagged Nassau grouper (n=177) captured and released in The Bahamas between 2014-2017 for genetic and telemetry studies (Sherman et al. unpubl. data). The red line represents the current minimum size limit and the dashed red line denotes the mean size at which Nassau grouper first migrate in The Bahamas.

Photo 4. Undersized Nassau grouper at a fish landing site in Eleuthera.
Photo Credit: Aaron Shultz

Establishment & Implementation of New National Regulations

1. Ban use of fish traps/pots around FSAs during the spawning season

Justification for new regulation: Baited fish traps or pots are the gear type primarily used at FSAs (Photo 5). Deployment of these traps at or within the vicinity of FSAs increases the probability that Nassau grouper migrating to and from FSAs to home reefs during the spawning season will be captured. Moreover, traps that become lost at sea are responsible for "ghost fishing", which also contributes to reducing Nassau grouper biomass. Given the prevalence for fish traps used in The Bahamas, fishers should be encouraged to build or purchase biodegradable traps. Additionally, we suggest **restricting deployment of traps throughout The Bahamas to ≤30 ft** during the spawning season.

Photo 5. Nassau groupers caught in a fish trap at an active FSA off Long Island, Bahamas
Photo credit: Krista Sherman

The use of compressors/hookahs is presently restricted to depths between 9.1-18.3 m (30-60 ft), which means this gear type should NOT be used at any FSA as these sites are typically ≥30.48 m (≥100 ft). Because Nassau grouper are solitary dwelling species outside of the reproductive season, the continued use of this gear type during non-breeding periods, is unlikely to lead to overharvest of the species.

2. Protect multi-species FSAs

Justification for new regulation: FSAs may be utilized by other fish species, e.g. black grouper (*Mycteroperca bonaci*) throughout the year. Spawning aggregation fishing of any species is not a sustainable practice and if unchecked can result in population collapse (Sadovy de Mitcheson and Colin 2012). Multi-species FSAs are important and unique ecological features that warrant protection. There are a few reported multi-species FSAs in The Bahamas, which are used by Nassau

grouper, black grouper (*Mycteroperca bonaci*) and other species (e.g. mutton snapper, *Lutjanus analis*).

3. **Establish maximum size limit**

Justification for new regulation: It is important to preserve size structure to maintain healthy fish populations (Hixon et al. 2013). Fishers are already reporting a decrease in both abundance and size of Nassau grouper, which is typically an indication of overfishing (Cheung et al. 2013; Wise et al. unpubl. data). Protecting juveniles, subadults and experienced, large sexually mature fish will be important for assisting with replenishing fish stocks. However, increasing the current minimum size limit to ≥54 cm (21.3 in) TL, should be prioritized to prevent further harvest of subadults or inexperienced spawners.

Strategic Surveillance & Enforcement

We recommend implementation of the following to assist with improving surveillance and enforcement for Nassau grouper:

1. RBDF patrols of active FSAs 3-4 days around the full moon during December and January (or peak spawning months moving forward)
2. Fisheries Officer inspections of local fish houses, landing sites and mailboats one week before the closed season and 2x per week during week of full moon during spawning season
3. Establish and enforce fines for grouper species landed without skin intact – proceeds to assist with on-going surveillance and enforcement
4. Increase and enforce fines for illegally caught and purchased fish – proceeds to assist with on-going surveillance and enforcement
5. Fishermen have expressed interested in assisting with enforcement to deter foreign poaching (illegal fishing). Develop a system (e.g. hotline, app, etc.) to encourage cooperation and facilitate faster reporting and response by enforcement officers
6. Publicise when fines and arrests have been made in association with IUU fishing.

Bahamas National Protected Area System (BNPAS) expansion

The following FSAs have been identified as important based on spawning stock biomass, genetic diversity and genetic connectivity, acoustic telemetry, and/or multi-species use:

1. Cay Verde (Ragged Island)
2. Hopetown (Abaco)
3. Hail Mary (between Little Exuma and Long Island)
4. Hole in the Wall (Abaco)
5. Tinker Rocks (Andros)
6. Newton's Cay (Long Island)
7. Grouper Hole (Eleuthera)
8. Little Egg Island (Eleuthera)
9. Tommy Sound (Eleuthera).

Other FSAs will be added to this list as data become available. If possible, incorporating these areas into the BNPAS should be considered. Additionally, the continental shelf of the Exuma Sound and Tongue of the Ocean (TOTO) represent important adult migratory and larval dispersal corridors, connecting natal reefs with FSAs. Drifter tracks and current trajectories indicate that larvae and pelagic juveniles are likely to spend a portion of their life cycle (35-50 days) in pelagic waters prior to recruiting to appropriate nursery habitats. Anthropogenic activities that have the potential to significantly alter water quality in the Exuma Sound and TOTO should be strictly regulated.

Monitoring & Evaluation

Monitoring and evaluation are critical components of the adaptive management cycle, which is an iterative learning process (Fig. 16).

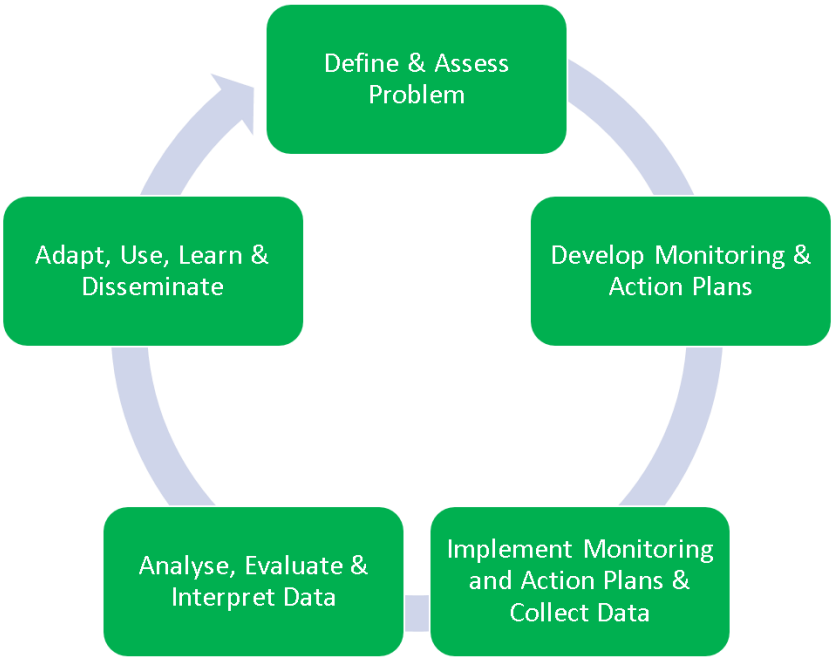


Figure 16. Simplified adaptive management cycle modified from The Open Standards for the Practice of Conservation.

To assess spatial and temporal changes in the health of Nassau grouper (i.e. abundance, size distribution, density, spawning stock biomass, genetic diversity and habitat use), both fishery-independent and fishery-dependent data are required (Table 5). These data should be collected routinely as they are critical for understanding population trends over time and applying appropriate management actions, which should be reviewed and amended as necessary (Appendices B-D).

Table 5. Data required for monitoring the status of Nassau grouper.

Fishery-independent Data	Fishery-dependent Data
• Spawning stock biomass (FSA monitoring) • Density of Nassau grouper (across all	• Commercial landings

habitats) • Size distribution/structure (across all habitats) • Genetic diversity (H_E, H_O, A_R) • Effective population size, N_e • Sighting frequency data (i.e. roving diver surveys) • Habitat use (e.g. tagging, stable isotope analysis) • Sex ratios	• CPUE from commercial, recreational and subsistence fisheries

Education, Outreach and Advocacy

Considerable efforts have been undertaken to improve education and outreach for Nassau grouper conservation in The Bahamas. To further advance these initiatives, we recommend developing an expert-led communication strategy and plan, strengthening the electronic exchange of materials, increased use of social media channels, and executing a holistic targeted national campaign to promote regulatory compliance and sustainable consumption for **all** harvested marine species. Cultivating and/or strengthening a rapport with key representatives from the fishing community should assist with transmission issues.

Assessing Management Effectiveness

This conservation plan has four specific objectives along with actions to rebuild and monitor the Bahamian Nassau grouper population while also eliminating or managing the threats that compromise its recovery. The first objective is to increase Nassau grouper density and spawning stock biomass. The second objective is to establish sustainable harvest regulations to promote a healthy fishery. Reducing anthropogenic threats, which negatively impact the species is the third objective. The fourth and final objective is to maintain and/or improve critical marine habitats for all life stages (e.g. mangroves, coral reefs, spawning sites). To promote management effectiveness, measurable criteria should be used to assess whether the recovery objectives have been satisfactorily met. To achieve objectives, DMR is encouraged to strategically collaborate with local law enforcement organizations (e.g. RBDF, RBPF), local government, scientists, fishers, NGOs, regional networks and other relevant stakeholders through an adaptive management process (Figs. 16 and 17). The specific actions and activities required to accomplish these objectives are outlined below.

Recommended Recovery Actions

- ✓ Establish and implement surveillance programme for targeted enforcement of active FSAs and no-take MPAs
- ✓ Revise Fisheries Act based on current scientific data
- ✓ Protection of critical Nassau grouper habitats through MPA and marine reserve designation
- ✓ Continued Nassau grouper FSA verification and monitoring (see Appendix B)
- ✓ Continued native reef fish monitoring for key habitats (e.g. coral reefs, mangroves and seagrass beds)
- ✓ Establish national sustainable seafood consumption campaign
- ✓ Assess physiological responses of Nassau grouper to climate change related stressors

- ✓ Support restoration programmes for habitats used across all life stages
- ✓ Stringent monitoring of coastal development activities to minimize pollution and damage to the benthos, nursery and pelagic habitats
- ✓ Sustained lionfish removal in critical marine habitats
- ✓ Follow-through with "Early detection and rapid response" for marine IAS (see NISS)

Warning Indicators

The following indicators can be used to help monitor and guide management decisions to assist with promoting recovery for Nassau grouper:

↓CPUE
↓Commercial landings
↓Nassau grouper density/biomass on reefs
↓Nassau grouper recruitment and density in nursery habitats
↓Nassau grouper FSA abundance & biomass
↓Nassau grouper effective population size (N_e) & genetic diversity
↑Habitat degradation and loss
↑Invasive species (e.g. lionfish) abundance/density
↑Pollution (e.g. eutrophication, oil spills, etc.).

Recovery Criteria

1. Demographic Criterion

- Protection of 50% minimum nursery habitats
- Protection of 20% minimum reef and hardbottom habitats
- Protection of 5% minimum of pelagic habitats
- Protection & enforcement of **all active FSAs**

2. Threat-based Criterion

- 85-90% reduction in FSA fishing (e.g. decreased number of traps at FSAs)
- 85-90% decrease in the capture of undersized fish (i.e. <54 cm TL)

3. Management Effectiveness Criterion

- Closed season actively enforced with emphasis on known active FSAs
- Enforcement officers receive adequate and regular training (minimum of once per year)
- Increased resources available for consistent FSA monitoring
- Enforcement at point-of-sale areas (e.g. landing sites, fish houses)

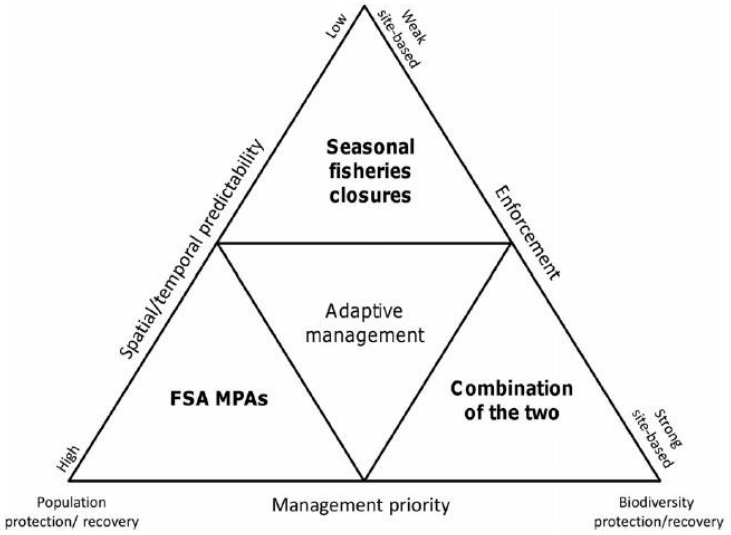


Figure 17. Conceptual model of adaptive management process for FSAs. Image from Grüss et al. 2014.

A summary of management objectives with linked tasks and associated timelines are provided in Table 6.

Implementation Timeline

The restoration of threatened and endangered species requires timely implementation of species-specific recovery plans to protect species and essential habitats (Taylor et al. 2005). **Immediate action** is required to stabilize remaining populations and facilitate the process of recovery because: 1) Nassau grouper FSAs are unlikely to recover once extirpated, 2) fish have experienced dramatic reductions in effective population size (N_e), 3) commercial fisheries are declining, 4) fish density/biomass in coral reef habitats is decreasing, and 5) on-going anthropogenic threats to FSAs and marine habitats (e.g., overfishing and climate change) persist. The technical advisory team will liaise closely with relevant management authorities and the Nassau grouper working group to fund and implement the national management plan.


Epinephelus striatus
"The Nassau Grouper"

Table 6. Five-year Implementation timeline with associated tasks and responsible entities.

Tasks	Responsible Organization(s)/Individual(s)	Supporting Organization(s)/Individual(s)	Year 1	Year 2	Year 3	Year 4	Year 5	On-going
Secure Funding								
Identify possible funders	DMR & Scientists	National & international NGOs						
Write grants/funding proposals to support: research & monitoring, outreach & advocacy, capacity building	Scientists	National & international NGOs						
Leverage local organizations/agencies to provide in-kind support (as appropriate)	National NGO	National & international NGOs						
Engage Stakeholders								
Develop communication plan/strategy	Consultant	National NGOs						
Conduct fishermen focus group meetings on major fishing islands: 1) acquire information (i.e. incorporate local knowledge into research) 2) share information/increase awareness	DMR/Consultant	National NGOs						
Workshops/Community Meetings/Public Lecture Series for: policy-makers, restaurants, processing plants, fish houses, general public)	National NGO	National NGOs						
Create & Implement Restaurant Reward Program to promote sustainable seafood	Consultant	National NGOs						

Develop targeted stakeholder materials/refine existing materials to 1) raise awareness (importance, vulnerability, status) & 2) advocate for sustainable fishery	Consultant	National & international NGOs						
Evaluate effectiveness of communication/outreach initiatives	Consultant	National NGOs						
Revise communication/outreach initiatives based on evaluation report	Consultant	National NGOs						
Research & Monitoring								
Identify active Nassau grouper FSAs	Scientists	National NGOs						
Identify multi-species FSAs	Scientists	National NGOs						
Stock assessment analysis	Scientists	GOB						
Standardize & implement consistent monitoring program to assess abundances & evaluate status/health of active FSAs	Scientists	National NGOs						
Establish important source-sink areas, migratory corridors and establish mechanisms influencing contemporary patterns of genetic connectivity	Scientists	National NGOs						
Prioritise FSAs for management	Scientists	GOB & National NGOs						
Complete economic evaluation for the species	Consultant	Scientists						
Incorporate biological and socioeconomic research into Nassau Grouper Conservation/Sustainable Fishery	Scientists	GOB						

Management Plan as new information becomes available								
Re-evaluate Fishery Regulations/Policies, Enforcement & Governance								
Strengthen institutional relationships & support among enforcement agencies	DMR	BNT, RBDF						
Engage fish processing plants to comply with fishery regulations (i.e. not purchasing undersized Nassau grouper or Nassau grouper caught during the closed season)	DMR	National NGOs						
Revise Fisheries Act or create FSA Management Act based on management plan recommendations	DMR	AG's office						

Estimated Costs

Estimated expenditures associated with this management plan are based on projections for the next five years.

Recovery Costs
Monitoring & Evaluation = $2,000,000
Surveillance & Enforcement = $5,000,000
Education, Outreach & Advocacy = $500,000
Estimated Total = $7.5 million

Conclusion

Available ecological and socioeconomic data highlight the urgency of improving conservation management for Nassau grouper in The Bahamas. Existing management frameworks need to be amended to promote sustainability of the commercial fishery and provide adequate protection to inexperienced spawners and spawning stocks through strategic enforcement, species and habitat monitoring, and effective public engagement. Recommendations for addressing these issues and assessing management strategies should be revised and adapted as new information becomes available. Regional and international cooperation (e.g., FAO WECAFC Spawning Aggregations Working Group) is strongly encouraged to assist with achieving recovery objectives.

This document was prepared by:

Authors	Affiliations	Contact Information
Krista D. Sherman, PhD Research Associate	University of Exeter Biosciences Geoffrey Pope Building Stocker Road EX4 4QD, UK John G. Shedd Aquarium Daniel P. Haerther Center for Conservation and Research 1200 South Lake Shore Drive Chicago Illinois 60605-2490, USA Bahamas National Trust Science & Policy P. O. Box N-4105 Nassau, Bahamas	krista.daniellesherman@gmail.com kds204@exeter.ac.uk
Craig Dahlgren, PhD Executive Director, PIMS Senior Science Advisor	Perry Institute for Marine Science P. O. Box 435 Route 100, Suite 1 Waitsfield VT, 05673 USA Bahamas National Trust Science & Policy P. O. Box N-4105 Nassau, Bahamas	cdahlgren@perryinstitute.org craigdahlgren@yahoo.com
Lindy Knowles, MSc Senior Science Officer	Bahamas National Trust Science & Policy P. O. Box N-4105 Nassau, Bahamas	lknowles@bnt.bs

Acknowledgements

This research was a collaborative initiative between the University of Exeter, John G. Shedd Aquarium, Perry Institute for Marine Science and Bahamas National Trust endorsed by the Department of Marine Resources. Research that supported the development of this plan was funded by the John G. Shedd Aquarium, University of Exeter, Save Our Seas Foundation, the Lyford Cay Foundation via the Shirley Oakes Butler Charitable Trust, Association of Zoos and Aquariums, Paul M. Angell Foundation, Ignacio de La Rocha, Krista Sherman, Disney Conservation Fund and Moore Bahamas Foundation.

In-kind contributions for various aspects of the research were provided by the Bahamas National Trust, Marine Aquarium Operations Department of Atlantis and Perry Institute for Marine Science. Drifters were donated by Dr. Claire Paris (University of Miami). Tremendous thanks are also extended to the crew of the R/V Coral Reef II, Shedd Aquarium staff and to numerous local and international volunteers that have assisted with this project. In-country volunteers included individuals from the Bahamas National Trust, Cape Eleuthera Institute, Atlantis, BREEF, The Nature Conservancy, Friends of the Environment and the Exuma Foundation.

We thank Dr. Lester Gittens (Department of Marine Resources), Professor Charles Tyler and Dr. Jamie Stevens (University of Exeter) for technical reviews of an earlier draft of this management plan.

Financial Sponsors

References

Aguilar-Perera, A (2006) Disappearance of a Nassau grouper spawning aggregation off the southern Mexican Caribbean coast. Marine Ecology Progress Series 327:289–296

Aguilar-Perera A, Aguilar-Davila W (1996) A spawning aggregation of Nassau grouper *Epinephelus striatus* (Pisces: Serranidae) in the Mexican Caribbean. Environmental Biology of Fishes 45:351–361.

Albins MA, Hixon MA, Sadovy Y (2009) Threatened fishes of the world: *Epinephelus striatus* (Bloch, 1792) (Serranidae). Environmental Biology of Fishes 86:309–310

Archer SK, Allgeier JE, Semmens BX, Heppell SA, Pattengill-Semmens CV, Rosemond AD, Bush PG, McCoy CM, Johnson BC, Layman CA (2015) Hot moments in spawning aggregations: implications for ecosystem-scale nutrient cycling. Coral Reefs 34: 19–23

Archer SK, Heppell SA, Semmens BX, Pattengill-Semmens CV, Bush PG, McCoy CM, Johnson BC (2012) Patterns of color phase indicate spawn timing at a Nassau grouper *Epinephelus striatus* spawning aggregation. Current Zoology 58: 73–83

Baisre JA and Paez J (1981) Los recursos pesqueros del archipelago Cubano. WECAF Stud 8, 79p

Bolden SK (2000) Long-distance movement of a Nassau grouper (*Epinephelus striatus*) to a spawning aggregation in the central Bahamas. Fishery Bulletin 98:642–645

Bush PG, Lane ED, Ebanks-Petrie GC, Luke K, Johnson B, McCoy, C, Bothwell J, Parsons E (2006) The Nassau Grouper Spawning Aggregation Fishery of the Cayman Islands – An Historical and Management Perspective. Proceedings of the Gulf and Caribbean Fisheries Institute 57:515–524

Camp EF, Lohr KE, Barry SC, Bush PG, Jacoby SA, Manfrino C (2013) Microhabitat associations of late juvenile Nassau Grouper (*Epinephelus striatus*) off Little Cayman. Bulletin of Marine Science 89: 571–581

Canty SWJ and Box SJ (2013) The last of the aggregations: validation of an extant grouper spawning aggregation in Honduras. Proceedings of the Gulf and Caribbean Fisheries Institute 66:381

Carpenter KE, Claro R, Cowan J, Sedberry G, Zapp-Sluis M (2015) *Epinephelus striatus*. The IUCN Red List of Threatened Species 2015: e.T7862A70324790. Downloaded on 14 March 2018

Carter J, Marrow GJ, Pryor V (1991) Aspects of the ecology and reproduction of Nassau grouper, *Epinephelus striatus*, off the coast of Belize, Central America. Proceedings of the Gulf and Caribbean Fisheries Institute 43: 65–111

Cheung WWL, Sadovy Y, Braynen MT, Gittens LG (2013) Are the last remaining Nassau grouper *Epinephelus striatus* fisheries sustainable? Status quo in the Bahamas. Endangered Species Research 20: 27–39

Claro R and Lindeman KC (2003) Spawning aggregation sites of snapper and grouper species (Lutjanidae and Serranidae) on the insular shelf of Cuba. Gulf and Caribbean Research 14: 91–106

Coleman FC, Koenig CC, Collins LA (1996) Reproductive styles of shallow-water groupers (Pisces Serranidae) in the eastern Gulf of Mexico and the consequences of fishing spawning aggregations. Environmental Biology of Fishes 47:129–141

Colin PL (1992) Reproduction of the Nassau grouper, *Epinephelus striatus*, (Pisces: Serranidae) and its relationship to environmental conditions. Environmental Biology of Fishes 34:357–377

Colin PL (2012) Aggregation Spawning: Biological aspects of the early life history. In: Sadovy de Mitcheson Y, Colin PL (eds) Reef fish spawning aggregations: biology, research and management. Springer, New York, NY, p 191−224

Colin PL, Laroche WA, Brothers EB (1997) Ingress and Settlement in the Nassau grouper, *Epinephelus striatus* (Pisces: Serranidae), with Relationship to Spawning Occurrence Bulletin of Marine Science 60:656–667

Cornish A, Eklund A-M (2003) *Epinephelus striatus*. The IUCN Red List of Threatened Species. Version 2014.2. <www.iucnredlist.org>. Downloaded on 23 October 2014.

Craig MT, Heemstra PC, Sadovy YJ (2011) Groupers of the world: a field and market guide. National Inquiry Services Centre (NISC), Grahamstown

Cushion N, Cook M, Schull J, Sullivan-Sealey K (2008) Reproductive classification and spawning seasonality of *Epinephelus striatus* (Nassau grouper), *E. guttatus* (red hind) and *Mycteroperca venenosa* (yellowfin grouper) from The Bahamas. Proceedings of the 11th International Coral Reef Symposium, Ft Lauderdale, Florida 22: 994−998

Dahlgren CP, Buch K, Rechisky E, Hixon MA (2016a) Multiyear tracking of Nassau grouper spawning migrations. Marine and Coastal Fisheries 8:522–535

Dahlgren CP, Eggelston DB (2001) Spatio-temporal variability in abundance, size and microhabitat associations of early juvenile Nassau grouper *Epinephelus striatus* in an off-reef nursery system. Marine Ecology Progress Series 217:145–156

Dahlgren CP, Kellison GT, Adams AJ, Gillanders BM and others (2006) Marine nurseries and effective juvenile habitats: concepts and applications. Marine Ecology Progress Series 312:291−295

Dahlgren C, Kramer PR, Lang J, Sherman K (2014) New Providence and Rose Island, Bahamas 2014 Coral Reef Report Card.

Dahlgren C, Sherman K, Lang J, Kramer PR, Marks K (2016b) Bahamas coral reef report card, Vol. 1: 2011−2013. Nassau, Bahamas. http://www.agrra.org/wp-content/uploads/2016/05/Bahamas-2016-Coral-Reef-Report-Card.pdf

de Castro MCT, Fileman TW, Hall-Spencer JM (2017) Invasive species in the Northeastern and Southwestern Atlantic Ocean: A review. Marine Pollution Bulletin 116:41−47

Domeier ML (2012) Revisiting Spawning Aggregations: Definitions and Challenges. In: Sadovy de Mitcheson, Y. and Colin, P. L. (2012) Reef Fish Spawning Aggregations: Biology, Research and Management. Vol. 35. Springer, 1-20.

Eggleston DB (1995) Recruitment in Nassau grouper *Epinephelus striatus*: post-settlement abundance, microhabitat features, and ontogenetic habitat shifts. Marine Ecology Progress Series 124: 9–22

Eggleston DB, Grover JJ, Lipcius RN (1998) Ontogenetic diet shifts in Nassau grouper: trophic linkages and predatory impact. Bulletin of Marine Science 63: 111–126

Ehrhardt NM, Deleveaux VKW (2007) The Bahamas' Nassau grouper (*Epinephelus striatus*) fishery – two assessment methods applied to a data – deficient coastal population. Fisheries Research 87: 17–27

Froese R, Pauly D (eds) (2014) FishBase. www.fishbase.org (accessed on 23 October 2014)

Grover JJ (1993) Trophic ecology of pelagic early-juvenile Nassau grouper, *Epinephelus striatus*, during an early phase of recruitment into demersal habitats. Bulletin of Marine Science 53: 1117–1125

Grover JJ, Eggleston DB, Shenker JM (1998) Transition from pelagic to demersal phase in early-juvenile Nassau grouper, *Epinephelus striatus*: pigmentation, squamation and ontogeny of diet. Bulletin of Marine Science 62: 97–113

Heemstra PC, Randall JE (1993) FAO species catalogue, Vol. 16. Groupers of the world (family Serranidae, subfamily Epinephelinae): an annotated and illustrated catalogue of the grouper, rockcod, hind, coral grouper, and lyretail species known to date. FAO Fisheries Synopsis No. 125, Food and Agriculture Organization of the United Nations, Rome

Heppell SA, Semmens BX, Pattengill-Semmens CV, Bush PG, Johnson BC, McCoy CM, Paris C, Gibb J, Heppell SS (2009) Tracking potential larval dispersal patterns from Nassau grouper aggregation sites: evidence for local retention and the 'importance of place'. Proceedings of the Gulf and Caribbean Fisheries Institute 61: 325–327

Heppell SA, Semmens BX, Pattengill-Semmens CV, Bush PG, Johnson BC, McCoy CM, Gibb J, Heppell S (2011) Oceanographic patterns associated with Nassau grouper aggregation spawn timing: shifts in surface currents on the nights of peak spawning. Proceedings of the Gulf and Caribbean Fisheries Institute 63: 152–154

Heppell SA, Semmens BX, Archer SK, Pattengill-Semmens CV, Bush PG, McCoy CM, Heppell SS, Johnson BC (2012) Documenting recovery of a spawning aggregation through size frequency analysis from underwater laser callipers measurements. Biological Conservation 155:119–127

Heyman WD, Kjerfve B (2008) Characterization of transient multi-species reef fish spawning aggregations at Gladden Spit, Belize. Bulletin of Marine Science 83: 531–551

Heyman W, Requeña N (2002) Status of multi-species spawning aggregations in Belize. The Nature Conservancy, Punta Gorda

IUCN (International Union for Conservation of Nature) (2015) IUCN red list of threatened species, Version 2015-3. www.iucnredlist.org (accessed on 03 July 2015)

Kadison E, Nemeth RS, Blondeau J, Smith T, Calnan J (2010) Nassau Grouper (*Epinephelus striatus*) in St. Thomas, US Virgin Islands, with Evidence for a Spawning Aggregation Site Recovery. In: Proceedings of the Gulf and Caribbean Fisheries Institute 62:273–279

Kobara S, Heyman WD (2008) Geomorphometric patterns of Nassau grouper (*Epinephelus striatus*) spawning aggregation sites in the Cayman Islands. Marine Geodesy 31:231–245

Ma KY, Craig MT, Choat JH, van Herwerden L (2016) Molecular biogeography of groupers: Clade diversification patterns and processes. Molecular Phylogenetics and Evolution 100:21–30

Marks KW, Lang JC (2016) AGRRA Summary Products, version (2016-08). Available online <http:www.agrra.org/data-explorer/explore-summary-products/ >

Nemeth RS, Kadison E, Herzlieb S, Blondeau J, Whiteman EA (2006) Status of a Yellowfin (*Mycteroperca venenosa*) Grouper Spawning Aggregation in the US Virgin Islands with Notes on Other Species. Proceedings of the Gulf and Caribbean Fisheries Institute 57: 543–558

O'Farrell S, Bearhop S, McGill RAR, Dahlgren CP, Brumbaugh DR, Mumby PJ (2014) Habitat and body size effects on the isotopic niche space of invasive lionfish and endangered Nassau grouper. Ecosphere 5:123, DOI 10.1890/es14-00126.1

Olsen DA, LaPlace JA (1979) A study of a Virgin Islands grouper fishery based on a breeding aggregation. In: Proceedings of the Gulf and Caribbean Fisheries Institute 31:130–144

Powell AB, Tucker Jr. JW (1992) Egg and Larval Development of Laboratory-Reared Nassau grouper, *Epinephelus striatus* (Pisces, Serranidae) Bulletin of Marine Science 50: 171–185

Ray GC (2000) Investigations of Nassau grouper breeding aggregations at High Cay, Andros: implications for a conservation strategy. Final Report, Department of Fisheries, Nassau

Sadovy de Mitcheson Y, Craig MT, Bertoncini AA, Carpenter KE, Cheung WWL, Choat JH, Cornish AS, Fennessy ST, Ferreira BP, Heemstra PC, Liu M, Myers RF, Pollard DA, Rhodes KL, Rocha LA, Russell BC, Samoilys MA, Sanciangco J (2013) Fishing groupers towards extinction: a global assessment of threats and extinction risks in a billion dollar fishery. Fish and Fisheries 14:119–136

Sadovy de Mitcheson YS, Cornish A, Domeier M, Colin PL, Russell M, Lindeman KC (2008) A Global Baseline for Spawning Aggregations of Reef Fishes. Conservation Biology 22:1233–1244

Sadovy Y, Colin PL (1995) Sexual development and sexuality in the Nassau grouper. Journal of Fish Biology 46:961–976

Sadovy Y, Domeier M (2005) Are aggregation-fisheries sustainable? Reef fish fisheries as a case study. Coral Reefs 24:254–262

Sadovy Y, Eklund AM (1999) Synopsis of Biological Data on the Nassau Grouper, *Epinephelus striatus* (Bloch, 1792) and the Jewfish, *E. itajara*, Lichtenstein, 1822. 1–64

Sadovy de Mitcheson, YS, Erisman B (2012) Fishery and Biological Implications of Fishing Spawning Aggregations, and the Social and Economic Importance of Aggregating Fishes In: Reef Fish Spawning Aggregations: Biology, Research and Management. Vol. 35. Springer, 225–284

Sala E, Ballesteros E, Starr RM (2001) Rapid Decline of Nassau Grouper Spawning Aggregations in Belize: Fishery Management and Conservation Needs. Fisheries 26:23–30

Schärer MT, Nemeth MI, Appeldoorn RS (2010) Protecting a multi-species spawning aggregation at Mona Island, Puerto Rico. Proceedings of the Gulf and Caribbean Fisheries Institute 62:252−259

Schärer MT, Rowell TJ, Nemeth MI, Appledoorn RS (2012) Sound production associated with reproductive behaviour of Nassau grouper *Epinephelus striatus* at spawning aggregations. Endangered Species Research 19:29−38

Schärer-Umpierre M, Nemeth R, Tuohy E, Clouse K, Nemeth M, Appledoorn RS (2014) Nassau grouper *Epinephelus striatus* fish spawning aggregations in the US Caribbean. Proceedings of the Gulf and Caribbean Fisheries Institute 66: 408−412

Schofield PJ, Morris Jr. JA, Akins L (2009) Field Guide to Nonindigenous marine fishes of Florida. NOAA Technical Memorandum. NOS NCCOS 92

Schofield PJ (2010) Update on geographic spread of lionfishes (Pterois volitans [Linnaeus, 1758] and P. miles [Bennet, 1828]) in the Western North Atlantic Ocean, Caribbean Sea and Gulf of Mexico. Aquatic Invasions 5 Supplement 1: S117−S122

Shenker JM, Maddox ED, Wishinski E, Pearl A, Thorrold SR, Smith N (1993) Onshore transport of settlement-stage Nassau grouper *Epinephelus striatus* and other fishes in Exuma Sound, Bahamas. Marine Ecology Progress Series 98, 31–43.

Sherman KD, Dahlgren CP, Stevens JR, Tyler CR (2016) Integrating population biology into conservation management for endangered Nassau grouper *Epinephelus striatus*. Marine Ecology Progress Series 554:263–280

Sherman KD, King RA, Dahlgren CP, Simpson SD, Stevens JR, Tyler CR (2017) Historical and contemporary anthropogenic activities influence genetic population dynamics of Nassau grouper (*Epinephelus striatus*) within The Bahamas. Frontiers in Marine Science 4:393. doi.org/10.3389/fmars.2017.00393

Sherman KD, Shultz AD, Dahlgren CP, Thomas C, Brooks E, Brooks A, Brumbaugh DR, Gittens L, Murchie K (In Press). Contemporary and emerging fisheries in The Bahamas – conservation and management challenges, achievements and future directions. Fisheries Management and Ecology DOI: 10.1111/fme.12299

Smith CL (1971) A revision of the American groupers: *Epinephelus* and allied genera. Bulletin of the American Museum of Natural History 146:67–242

Smith CL (1972) A spawning aggregation of Nassau grouper, *Epinephelus striatus* (Bloch). Transactions of the American Fisheries Society 101:257−261

Smith NS, Green SJ, Akins JL, Miller S, Côté (2017) Density-dependent colonization and natural disturbance limit the effectiveness of invasive lionfish culling efforts. Biological Invasions 19:2385−2399

Starr RM, Sala E, Ballesteros E, Zabala M (2007) Spatial dynamics of the Nassau grouper *Epinephelus striatus* in a Caribbean atoll. Marine Ecology Progress Series 343:239–249

Stump K, Dahlgren CP, Sherman KD, Knapp CR (2017) Nassau grouper migrations during full moon suggest collapsed historic fish spawning aggregation and evidence of undocumented aggregation. Bulletin of Marine Science 93:375–389

Thompson R and Munro JL (1978) Aspects of the biology and ecology of Caribbean reef fishes Serranidae (hinds and groupers). Journal of Fish Biology 12:115–146

Tucker JW Jr, Woodard PC (1991) Growth and development of domestic juvenile Nassau groupers. Proceedings of the Gulf and Caribbean Fisheries Institute 43:389−391

Whaylen L, Pattengill-Semmens CV, Semmens BX, Bush PG, Boardmand MR (2004) Observations of a Nassau grouper, *Epinephelus striatus*, spawning aggregation site in Little Cayman, Cayman Islands, including multispecies spawning information. Environmental Biology of Fishes 70:305−313

Whaylen L, Bush PG, Johnson BC, Luke KE and others (2007) Aggregation dynamics and lessons learned from five years of monitoring at a Nassau grouper (*Epinephelus striatus*) spawning aggregation in Little Cayman, Cayman. Proceedings of the Gulf and Caribbean Fisheries Institute 59:1−14

Watson, A. C., Siemann, L.A. and Hanlon, R.T. (2014) Dynamic camouflage by Nassau groupers *Epinephelus striatus* on a Caribbean coral reef. Journal of Fish Biology 85: 1634–1649

Appendices

Disclaimer: The recommendations outlined in this management plan do not necessarily reflect those of the Department of Marine Resources.

Appendix A. Diet composition of Nassau grouper (*Epinephelus striatus*).

Prey Taxa	Common Name	References
TELEOSTS		
Acanthuridae	**Surgeonfish**	
Acanthurus coeruleus	Blue tang	Sadovy and Eklund 1999
Acanthurus sp.	Surgeonfish sp.	Carter et al. 1994; Sadovy and Eklund 1999
Apogonidae	**Cardinalfish**	Sadovy and Eklund 1999
Atherinidae	**Silversides**	Sadovy and Eklund 1999
Balistidae	**Triggerfish**	
Balistes vetula	Queen triggerfish	Sadovy and Eklund 1999
Bothidae	Left-eye flounders	Sadovy and Eklund 1999
Carangidae	**Jacks**	Sadovy and Eklund 1999
Caranx ruber	Bar jack	Sadovy and Eklund 1999
Clupeidae	**Herrings, shads, sardines**	
Harengula clupeola	False herring	Sadovy and Eklund 1999
Harengula humeralis	Redear herring	Sadovy and Eklund 1999
Jenkinsia lamprotaenia	Dwarf round herring	Sadovy and Eklund 1999
Epinephelidae	**Groupers**	
Cephalopholis fulva	Coney	Sadovy and Eklund 1999
Epinephelus striatus	Nassau grouper	Sadovy and Eklund 1999
Gerreidae	**Mojarra (colloquially "Shad")**	
Geres cinereus	Yellowfin mojarra	Sadovy and Eklund 1999
Gobiidae	**Gobies**	
Coryphopterus sp.	Goby sp.	Sadovy and Eklund 1999
Haemulidae	**Grunts**	
Haemulon album	White margate	Sadovy and Eklund 1999
Haemulon aurolineatum	Tomtate	Sadovy and Eklund 1999
Haemulon flavolineatum	French grunt	Sadovy and Eklund 1999
Haemulon plumieri	White grunt	Sadovy and Eklund 1999
Haemulon sciurus	Bluestriped grunt	Sadovy and Eklund 1999
Haemulon sp.	Grunts	Carter et al. 1994; Sadovy and Eklund 1999
Holocentridae	**Squirrelfishes & Soldierfishes**	

Holocentrus rufus	Longspine squirrelfish	Sadovy and Eklund 1999
Holocentrus sp.	Squirrelfish sp.	Carter et al. 1994; Sadovy and Eklund 1999
Myripristis Jacobus	Blackbar soldierfish	Sadovy and Eklund 1999
Sargocentron vexillarium	Dusky squirrelfish	Sadovy and Eklund 1999
Labridae	**Wrasse**	
Clepticus parrae	Creole wrasse	Sadovy and Eklund 1999
Halichoeres bivvitatus	Slippery dick	Sadovy and Eklund 1999
Halichoeres garnoti	Yellowhead wrasse	Sadovy and Eklund 1999
Halichoeres sp.	Wrasse sp.	Carter et al. 1994; Sadovy and Eklund 1999
Xyrichtys sp.	Razorfishes	Sadovy and Eklund 1999
Lutjanidae	**Snapper**	
Lutjanus synagris	Lane snapper	Sadovy and Eklund 1999
Lutjanus sp.	Snappers	Carter et al. 1994; Sadovy and Eklund 1999
Ocyurus chrysurus	Yellowtail snapper	Sadovy and Eklund 1999
Monacanthidae	**Filefish**	
Cantherhines pullus	Orangespotted filefish	Sadovy and Eklund 1999
Monacanthus ciliates	Fringed filefish	Sadovy and Eklund 1999
Monacanthus sp.	Filefish sp.	Sadovy and Eklund 1999
Mullidae	**Goatfish**	
Pseudupeneus maculatus	Spotted goatfish	Sadovy and Eklund 1999
Muraenidae	**Eels**	
Enchelycore nigricans	Viper moray eel	Sadovy and Eklund 1999
Gymnothorax moringa	Spotted moray eel	Sadovy and Eklund 1999
Gymnothorax sp.	Moray eels	Sadovy and Eklund 1999
Muraena miliaris	Goldentail moray eel	Sadovy and Eklund 1999
Muraena sp.	Mediterranean moray eels	Sadovy and Eklund 1999
Ostraciidae	**Boxfish**	Sadovy and Eklund 1999
Lactophrys sp.	Trunkfishes	Sadovy and Eklund 1999
Pomacentridae	**Damselfish**	
Abudefduf saxatalis	Sergeant major	Sadovy and Eklund 1999
Chromis cyanea	Blue chromis	Sadovy and Eklund 1999
Chromis mutlinieata	Brown chromis	Sadovy and Eklund 1999
Microspathodon chrysurus	Yellowtail damselfish	Sadovy and Eklund 1999

Pomacentrus fuscus	Brazilian damselfish	Sadovy and Eklund 1999
Pomacentrus sp.	Damselfish sp.	Carter et al. 1994; Sadovy and Eklund 1999
Priacanthidae	**Bigeyes**	
Priacanthus cruentatus	Glasseye snapper	Sadovy and Eklund 1999
Scarinae	**Parrotfish**	
Scarus croicensis	Striped parrotfish	Sadovy and Eklund 1999
Scarus sp.	Parrotfishes	Carter et al. 1994; Sadovy and Eklund 1999
Scarus vetula	Queen parrotfish	Sadovy and Eklund 1999
Sparisoma aurofrentatum	Redband parrotfish	Sadovy and Eklund 1999
Sparisoma chrysopterum	Redtail parrotfish	Sadovy and Eklund 1999
Sparisoma rubripinne	Redfin parrotfish	Sadovy and Eklund 1999
Sparisoma sp.	Parrotfish sp.	Carter et al. 1994; Sadovy and Eklund 1999
Serranidae	**Seabass & Basslets**	
Hypoplectrus puella	Barred hamlet	Sadovy and Eklund 1999
Synodontidae	**Lizzardfish**	Sadovy and Eklund 1999
Synodus intermedius	Sand diver	Sadovy and Eklund 1999
Synodus sp.	Lizzardfish sp.	Sadovy and Eklund 1999
Urolophidae	**Round Rays**	
Urolophus jamaicensis	Yellow stingray	Sadovy and Eklund 1999
BIVALVES		
Barbatia cancellaria	Red-brown ark clam	Sadovy and Eklund 1999
Pelecypoda	Oysters, clams, mussels, cockles	Eggleston et al. 1998; Sadovy and Eklund 1999
CRUSTACEANS		
Amphipods	Amphipods	Grover et al. 1998; Dahlgren and Eggleston 2000
Caprellidae	Skeleton shrimp amphipod	Grover et al. 1998
Gammaridea	Gammaridean amphipods	Grover et al. 1998
Copepods		
Calanoida	Calanoid copepods	Grover 1993; Grover et al. 1998
Cyclopoida	Cyclopoid copepods	Grover et al. 1998
Harpacticoida	Harpacticoid copepods	Grover 1993; Grover et al. 1998
Poecilostomatoida	Poecilostomatoid copepods	Grover 1993; Grover et al. 1998

Siphonostomatoida	Siphonostomatoid copepods	Grover 1993
Crabs		
Calappa flammea	Flame box crab	Sadovy and Eklund 1999
Calappa gallus	Rough box crab	Eggleston et al. 1998; Sadovy and Eklund 1999
Calappa sp.	Box crabs	Eggleston et al. 1998; Sadovy and Eklund 1999
Cronius tumidulus	Sculling crab	Eggleston et al. 1998
Euryplax nitida	Glabrous broadface crab	Sadovy and Eklund 1999
Grapsids	Marsh or shore crabs	Sadovy and Eklund 1999
Macrocoelema diplacanthum	Decorator crab	Eggleston et al. 1998; Sadovy and Eklund 1999
Macrocoelema sp.	Crabs	Sadovy and Eklund 1999
Micropanope pusilla	Puffy mud crab	Eggleston et al. 1998; Sadovy and Eklund 1999
Micropanope sp.	Mud crab sp.	Eggleston et al. 1998; Sadovy and Eklund 1999
Mithrax cinctimanus	Banded clinging crab	Sadovy and Eklund 1999
Mithrax coryphe	Nodose clinging crab	Eggleston et al. 1998; Sadovy and Eklund 1999
Mithrax forceps	Red-ridged clinging crab	Eggleston et al. 1998; Sadovy and Eklund 1999
Mithrax sp.	Clinging crabs	Eggleston et al. 1998; Sadovy and Eklund 1999
Mithrax spinosissiums	Channel clinging crab	Eggleston et al. 1998
Mithrax verrucosus	Paved clinging crab	Sadovy and Eklund 1999
Paguristes depressus	Hermit crab	Carter et al. 1994; Grover et al. 1998
Panopeus sp.	Mud crab sp.	Eggleston et al. 1998; Sadovy and Eklund 1999
Petrochirus Diogenes	Giant hermit crab	Sadovy and Eklund 1999
Petrolisthes galathinus	Amphi-American porcelain crab	Sadovy and Eklund 1999
Petrolisthes sp.	Porcelain crabs	Eggleston et al. 1998
Pitho aculeate	Massive urn crab	Eggleston et al. 1998; Sadovy and Eklund 1999
Pitho sp.	Urn crabs	Eggleston et al. 1998
Portunus sebae	Ocellate swimming crab	Sadovy and Eklund 1999
Portunus ordwayi	Redhair swimming crab	Eggleston et al. 1998
Portunus sp.	Swimming crab	Eggleston et al. 1998; Grover et al. 1998
Stenorhynchus seticornis	Yellowline arrow crab	Sadovy and Eklund 1999
Euphausiids	Krill	Grover et al. 1998
Isopods		Carter et al. 1994; Eggleston et al. 1998
Rocinela signata	Isopod	Eggleston et al. 1998
Lobsters		Carter et al. 1994

Justitia longimana	West Indian furrow lobster	Sadovy and Eklund 1999
Leptostraca	Leptostracans	Grover et al. 1998
Ostracods	Ostracods	Grover et al. 1998
Shrimp		
Alpheus sp.	Snapping shrimp	Eggleston et al. 1998
Artemia sp.	Brine shrimp	Tucker and Woodward 1991
Carideans	Emperor shrimp sp.	Grover et al. 1998; Eggleston et al. 1998
Cumaceans	Comma or hooded shrimp	Grover et al. 1998
Mysids	Mysid shrimp	Grover 1993; Grover et al. 1998
Paneidae	Paneid shrimp	Eggleston et al. 1998
Synalpheus sp.	Snapping shrimp	Eggleston et al. 1998
Thor sp.	Cleaner shrimp	Eggleston et al. 1998
Trachycaris restricta	Cleaner shrimp	Eggleston et al. 1998
Stomatopods		Carter et al. 1994; Eggleston et al. 1998; Grover et al. 1998
Alima sp.	Mantis shrimp	Carter et al. 1994; Eggleston et al. 1998
Pseudosquilla sp.	Common mantis shrimp	Eggleston et al. 1998
Squilla sp.	Mantis shrimp	Eggleston et al. 1998
ROTIFERA	Rotifers	Tucker and Woodward 1991
TANAIDACEA	Tanaids	Grover et al. 1998
CHAETOGNATHS	Arrow worms	Grover et al. 1998
CILLIATES	Protozoans	Grover et al. 1998
DINOFLAGELLATES	Flagellate protists (marine phytoplankton)	Grover 1993
FORAMINIFERANS	Forams (single-celled protists)	Grover 1993
MOLLUSCS		
Cephalopoda	Octopi	Carter et al. 1994; Eggleston et al. 1998

Cerithium sp.	Sea snail	Eggleston et al. 1998
Fasciolaria tulipa	True tulip snail	Eggleston et al. 1998
Lobatus gigas	Queen conch	Eggleston et al. 1998
Loligo sp.	Squid	Eggleston et al. 1998
Pteropoda	Pelagic sea snails and slugs	Grover et al. 1998
Strombus sp.	Conch	Eggleston et al. 1998
POLYCHAETA	Polychaete worms	Grover et al. 1998
PYCNOGONIDS	Sea spiders	
Pycnogonida sp.	Sea spider sp.	Carter et al. 1994; Eggleston et al. 1998; Grover et al. 1998
UKNOWN		
Invertebrate eggs	Invertebrate eggs	Grover 1993
Fish larvae	Fish larvae	Grover 1993
Decapod larvae	Decapod larvae	Grover et al. 1998

Appendix B - Nassau grouper FSA survey monitoring protocol

Nassau grouper FSA Survey Protocol

- Record metadata - surveyor name(s), date, survey start and end time, depth, temp, GPS & site name/location. Make sure the GPS location is as close as possible to where fish are observed to aggregate vs. the location of the boat at anchor at the site, which may be 100 m or more away.

- In addition to taking GPS points, it's also useful to take lots of photos and videos.
- Compose sketch of the site and describe habitat and any interesting features of the site. This should be done with someone experienced at underwater navigation or mapping.

- If possible, have the boat do multiple passes for 1-2 km around the site recording depth and GPS coordinates every 100 m or so (more frequently is possible) for a large scale map of the area.

- Documenting populations at the site should be done throughout the day. Because numbers of fish and possibly locations of fish change throughout the day, surveys should be conducted as many times as possible during the day (every 1-2 hours if personnel, logistics and weather allow).
    - Roving diver surveys (RDS) to estimate total number of individuals in the SPAG
    - Record size range (TL in cm) of groupers observed
    - Estimate the proportion in each of the 4 colour phases (normal or barred, bicolor, white belly, dark)
    - Estimate proportion with distended abdomens
    - Note direction which the school is moving (if they're moving), and other behaviours
    - Conduct multiple (30 x 2 m) * belt transect surveys (if fish are near or on the bottom) to record density, colour phase, and size (TL nearest cm) for Nassau grouper

*NB - will depend on size of team, survey area and the available bottom time)

- 3-4 days before & 1-2 days after the full moon conduct dives closer to sunset to document spawning behaviour. This should include dives anywhere from 1hr before to ½ hour after sunset. So in addition to RDS:
    - record any spawning behaviour observed including chasing (no. fish and information about size, time and colour phase), false rushes and other behaviours
    - document time of spawning release, number of individuals involved, colour phases, size estimates

- Note occurrence of poaching or illegal fishing. Take pics, record names of vessels (if possible).
    - number of boats per day/night
    - gear used & number of traps
    - numbers of fish in traps (release fish from traps if possible)

Nassau grouper Fin Clip Sampling Protocol

Live Fish Sampling: To ensure robust genetic analysis of a population, ideally samples of **50** fish should be collected from each site. Collected (fin clip) sample volume should NOT exceed 1/20th of the 2ml tube.

1. Cut ~5mm x 5mm fin clip from anesthetized (MS 222 buffered bath) fish using a pair of scissors.
2. Use tweezers to deposit sample in screw top vials with 95% ETOH.
3. Rinse/Wash scissors and tweezers between each sample to avoid cross contamination.
4. In pencil, write fish ID, SL cm, TL cm, Wt. (kg) collection date, site information (i.e. island and location) on waterproof slip and insert into vial. Ensure vial is properly sealed.
5. Label outside of vial with fish ID number using alcohol-proof pen. If unavailable, record information on a piece of paper in regular pen and cover with clear scotch tape.
6. Record tube number, site metadata and fish meristics on master data sheet and transfer to Excel spreadsheet.

Dead Fish Sampling: If samples are obtained from dead fish it is important to ensure that the sample is taken within a few minutes of death.

1. Cut ~5mm x 5mm fin clip from fish using a pair of scissors.
2. Use tweezers to deposit sample in screw top vials with 95% ETOH.
3. Rinse/Wash scissors and tweezers between each sample to avoid cross contamination.
4. In pencil, write fish ID, SL cm, TL cm, Wt. (kg) collection date, site information (i.e. island and location) on waterproof slip and insert into vial. Ensure vial is properly sealed.
5. Label outside of vial with fish ID number using alcohol-proof pen. If unavailable, record information on a piece of paper in regular pen and cover with clear scotch tape.
6. Record the tube number, details of date and place of fish capture, SL cm and TL cm. When sending samples for analysis, include a data sheet with sampling details for each tube.

Sample Storage: Samples are to be kept and stored in a refrigerator at all times until delivery to the Exeter lab.

Appendix D – Nassau grouper Fishery Management Workshop Report

Nassau Grouper Fishery Management Workshop

Nassau Grouper (*Epinephelus striatus*) Photo: Keith Pamper © 2011

Workshop Report
New Providence, Bahamas
March 17th, 2016

Table of Contents

Nassau Grouper Fishery Management Workshop

Date: Thursday, March 17th, 2016

Time: 1:00-3:00 PM

Venue: Harry C Moore Library Bibliographic Instruction Classroom, College of The Bahamas

Workshop Summary

The Nassau grouper (*Epinephelus striatus*) is a cultural icon as well as an economically and ecologically key marine fish species for The Bahamas. Approximately $1 million is generated through annual landings, and the fishery supports thousands of livelihoods throughout the archipelago. The species is at risk from a range of threats, most notably overfishing and illegal aggregation fishing, which threaten the long-term viability of the fishery.

Research has shown that fishing on spawning aggregations has decimated Nassau grouper populations regionally by as much as 90% over the past three decades. It is listed as endangered on the IUCN Red List and is a candidate species to be listed for special protection under the United States Endangered Species Act (ESA). The Bahamas government's decision in 2015 to impose a permanent annual three-month seasonal closure for grouper fishing is a positive step towards protecting this at-risk species. However, a multi-faceted approach is required to effectively manage the fishery because management strategies to date have not been effective in reversing the decline of Nassau grouper stocks.

In 2013, the Bahamas National Trust (BNT) hosted a Nassau Grouper Conservation Strategy Workshop to discuss ways to promote recovery for grouper populations. Both biological or ecological and sociocultural considerations were outlined. Specific research needs were identified and prioritized that would form the foundation for a science-driven conservation plan for the Bahamian Nassau grouper fishery. Under the theme "Reversing the Decline", a Nassau Grouper Fishery Management Workshop was held during the Bahamas Natural History Conference 2016 to outline key components and promote the establishment of a comprehensive national Nassau grouper sustainable management plan for The Bahamas (see workshop objectives).

Workshop introductions were led by Krista Sherman and meeting norms were established for the session. This was followed by a brief presentation by K. Sherman to outline previously agreed upon priorities and strategies that were discussed during the 2013 Nassau Grouper National Conservation Strategy Meeting. Dr. Kristine Stump and Casuarina McKinney-Lambert provided updates on research and education/outreach activities respectively toward

achieving 2013 goals. Information about the pilot Nassau grouper aquaculture project that is currently underway at Tropic Seafood was shared by Jon Chaiton. Michael Braynen delivered the final presentation on current fishery regulations and enforcement in The Bahamas.

These presentations set the stage for a structured break-out session with stakeholders. The discussions that followed were used to gather additional input about the status of the fishery as well as advancing existing and future policies to promote recovery of Nassau grouper populations in The Bahamas. Participants were divided into two smaller groups to complete a SWOT analysis[1] of the Nassau grouper fishery. Notes from each group were recorded by facilitators. Specifically, each group addressed the following questions (which were also provided via email a week prior to the workshop):

1. How would you characterize/describe (e.g. status, primary gears used, no. of people involved, etc.) the Nassau grouper fishery in your island? in The Bahamas?
2. In your opinion what are the strengths/benefits of the Nassau grouper fishery?
3. How would your rank/prioritize these strengths/benefits?
4. In your opinion what are the threats/issues facing the Nassau grouper fishery?
5. How would you rank/prioritize these threats/issues?
6. Do you have any recommendations on how these threats/issues can be addressed?

After reporting out from the small group exercises to the larger group, there was insufficient time to completely address workshop objectives 3 and 4, which were to identify short and long-term goals to promote the development of a comprehensive Nassau grouper management and conservation strategy for The Bahamas, and individuals/organizations and timelines to support various components of the national conservation plan (see workshop objectives).

Anticipated outcomes of this workshop included the formation of a multi-sector working group and Nassau grouper technical advisory committee comprised of policy-makers, law enforcement officials, fishermen, marine resource managers, scientists, non-governmental organizations (NGOs) and the private sector. It is envisioned that these groups will work together to identify management alternatives and strategies to promote a sustainable Nassau grouper fishery for The Bahamas. Closing remarks were given by Dr. Craig Dahlgren. A summary of the minutes from the workshop is provided within this report. The participant list and agenda are provided as Appendix I and II, respectively.

[1]Please review spreadsheet for details from the SWOT Analysis.

Workshop Objectives:

1) Status update on national research, conservation and outreach priorities outlined in 2013 for Nassau grouper stocks in The Bahamas.
2) Summarize progress on Nassau grouper research in the country.
3) Identify short and long-term goals to promote the development of a comprehensive Nassau grouper management and conservation strategy for The Bahamas.
4) Identify individuals/organizations and timelines to support various components of the national conservation plan.

Workshop Minutes

Characterizations of the Nassau Grouper Fishery

Mr. Braynen confirmed that Nassau grouper are not exported from The Bahamas due to within-country demand fulfilling and/or surpassing supply. A variety of methods are used to capture groupers. These include hand-line fishing, free and compressor diving with spears, recreational drop lines, and pots or fish traps, which are the most frequently used method to catch groupers. Concerns were expressed over the status of the fishery from participants residing in different islands. In Eleuthera, there is no attention to regulations. All gears are being used even during the closed season. Harvesting of parrotfish has also increased as a substitute for Nassau grouper and other traditionally eaten fish (e.g. snappers). This emerging fishery is currently not regulated and is spreading throughout the country. Nassau grouper are consistently seen hitting the docks during the closed season in New Providence (e.g. Potter's Cay and Montague). Tourists are also reported to be fishing them year-round in areas such as Bimini. In Acklins, Nassau grouper are also being harvested year-round.

Key Challenges/Issues

"We can inform thousands of people, but are we already at the point of no return with the grouper population? How do we better enforce the laws already in place?" *– T. Thompson*

A number of challenges and issues relating to Nassau grouper conservation were brought-up and discussed. One of the key areas discussed was relating to enforcement, monitoring and compliance. A question was raised as to how we can enforce the laws we already have. Responses from the group included securing funding to strengthen the resources and capacity to assist with enforcement, monitoring/research, education and advocacy.

The following non-prioritized issues were raised during the workshop as barriers to Nassau grouper conservation:

- Inadequate enforcement – huge area to cover
- Non-compliance with current fishery regulations (e.g. illegal domestic and foreign fishing)
- Lack of effective education/outreach
- Lack of training and resources for Fisheries Officers and other law enforcement representatives
- Officers may choose not to enforce the law because fishermen know where they live and they are part of these communities
- Lack of administrative penalties for violating fishery regulations
- Reluctance of fishermen to reveal locations of other FSAs (e.g. High Cay was closed to fishing once this location was provided by fishermen)
- Scientific gaps with regards to status of Bahamian Nassau Grouper FSAs and stock size.

Recommendations

"The best way to communicate science to fishermen is to go to the fishing community and talk to the fishermen directly. Send teams to areas/islands because fishermen are not educated on the issue. Put signs up around the islands that say when seasons are closed". – *Dwain "Tall Boy" Bastian*

The group also discussed possible ways to overcome these challenges. With regards to enforcement, it was recommended that Fisheries Officers be sent to docks since it is cheaper than patrolling via boat. Fishermen also volunteered to use their boats to help with enforcement. However, Mr. Braynen advised that this would require a policy change to address liability issues. The Department of Marine Resources also has 12 new Fisheries Officers that will be assigned to various islands throughout the country. Fishermen noted that mailboats often have bags of grouper and these should also be inspected. Consensus from the group was that stricter penalties need to be implemented at an administrative level to act as a deterrent. Examples discussed included issuing tickets and increasing fines.

In terms of education and outreach, a suggestion was also made to have regular trainings for enforcement officers and other regulatory agencies so that they are aware of all fishery regulations (e.g. size limits, closed seasons, gear restrictions), locations of MPAs, boundaries, etc. NGOs are also willing to cross-post existing educational materials on other islands. However, new approaches need to be developed and tested to target market-demand issues. Recommendations included the development of materials for tourists, hotel associations, fish processing plants/vendors and restaurants as they help to create the demand for Nassau grouper.

It was also stated that we need to find better ways to engage fishermen. Fishermen advised that the best way to communicate science and relevant information to the local fishing communities is through one-on-one conservations, posting on informational boards at

landings sites and identifying and training a few fishermen that are willing to represent each island and educate other fishermen.

There is also a need to understand and address fisher's perceptions and concerns as these vary throughout the country. The viewpoints below are reflective of the fishermen who attended the workshop:

- Some fishermen believe that fish size has increased since the ban.
- Some fishermen think that Nassau groupers have spread out to other areas as some aggregations disappear, but new aggregations are forming in other areas.
- Spearing is bad because it "breaks up schools and attracts sharks", which is dangerous for fishermen. The method that should be practiced more is bringing live fish to the boat. This was done in the past and some fishermen believe that it should resume because it helps to limit the numbers of fish that can be caught at a time. Only fish that could fit into a live well were caught. "Divers would bring live fish to the boat. Hold fish under arm, stick under left fin with needle to release air then bring to the surface". Concern was still raised with regard to this method because it sounds like it occurred at during spawning aggregations.

Other points raised included the need to improve upon data collection including the quality and consistency of fishery-dependent data (e.g. landings) to better understand trends in the fishery. The fishery data collected does not accurately reflect all Nassau grouper landings and excludes recreational and subsistence fishery data. Increasing reports of sea turtles being harvested and sharks being killed also highlight the need for better "follow through" with law enforcement, education and outreach. Additional needs include addressing knowledge gaps relating to the status of spawning stocks (currently underway) and undertaking an economic evaluation of the Nassau grouper fishery. Other organizations or agencies that could potentially be contacted to assist with ongoing conservation efforts include the Royal Bahamas Defence Force (RBDF), Royal Bahamas Police Force (RBPF) Customs, Ministry of Tourism and the Maritime Authority.

Next Steps

Securing funding to complete the various tasks associated with Nassau grouper conservation management remains a top priority. Reducing this funding gap is likely to help address issues with increasing capacity and resources available for consistent research, FSA monitoring, fishery regulatory enforcement and expanding education and outreach programmes tailored to various groups. Next steps are to:

- Review and provide comments on the workshop report
- Provide feedback and prioritize the SWOT analysis
- Form a multi-sector Nassau grouper working group
- Assign a point-person within each agency to become a member of the Nassau grouper working group

- Identify possible sources of funding (actual and in-kind) for a socioeconomic evaluation of the fishery, research into the status and health of grouper stocks, and communications to key stakeholders and the general public about the closed season
- Incorporate “low-hanging fruit” activities into organizational workplans
- Complete a questionnaire that will be used to pilot test a national survey on the Nassau grouper fishery.

8

Sherman, Krista

Appendix I: Workshop Participants

Attendees

1. Michael Braynen (Director, Department of Marine Resources)
2. Eric Carey (Executive Director, Bahamas National Trust)
3. Casuarina McKinney-Lambert (Director, BREEF)
4. Jon Chaiton (Quality Assurance & Aquaculture Manager, Tropic Seafood)
5. Leroy Miller (Immigration Officer, Department of Immigration
6. Tavares Thompson (Fisherman & Entrepreneur, Andros)
7. Henry Bannister (Fisherman, Bahamas Commercial Fishers Alliance)
8. Dwain Bastian (Fisherman, New Providence)
9. Indira Brown (Assistant Fisheries Officer, Department of Marine Resources)
10. Lindy Knowles (Senior Science Officer, Bahamas National Trust)
11. Frederick Arnett (Conservation Practitioner, The Nature Conservancy)
12. Dr. Aaron Shultz (Scientist, Fisheries Conservation Foundation)
13. Candice Brittain (Applied Scientific Research Department Head, Cape Eleuthera Institute)
14. Angela Rosenberg (Director of Programs and Policies, International SeaKeepers Society)
15. Dr. Mark Bond (Consultant, Moore Charitable Foundation)

Facilitators

1) Krista Sherman (PhD Candidate, University of Exeter)
2) Dr. Kristine Stump (Postdoctoral Associate, Shedd Aquarium)
3) Dr. Craig Dahlgren (Senior Scientist, Bahamas National Trust)
4) Janeen Bullard (Environmental Specialist)

Apologies

1. Shenique Albury-Smith (The Nature Conservancy)
2. Kristin Williams (Friends of the Environment)
3. Catherine Booker (The Exuma Foundation)
4. Nick Rademaker (Harbourside Marine)
5. Dr. Valierre Deleveaux (BAMSI)
6. Mia Isaacs (Bahamas Marine Exporters Association)
7. Vanessa Haley-Benjamin (College of The Bahamas)

Appendix II – Agenda

Nassau Grouper Fishery Management Workshop

Date: Thursday, March 17th, 2016

Time: 1:00-3:00 PM

Venue: Harry C Moore Library Bibliographic Instruction Classroom, College of The Bahamas

Time	Topic	Name
1:00-1:05 PM	Welcome and Introductions	Krista Sherman (Research Scientist)
1:10-1:20 PM	Research Guided Policy-Making	Krista Sherman, Research Scientist
1:25-1:35 PM	Summary of Research Findings	Dr. Kristine Stump, Shedd Aquarium
1:40-1:50 PM	Education & Outreach	Casuarina McKinney-Lambert (BREEF)
1:55-2:00 PM	New Approaches to Commercial Fisheries	Jon Chaiton, Tropic Seafood
2:05-2:15 PM	Overview of Fishery Regulations & Enforcement	Michael Braynen, Director of Marine Resources
2:20-2:50 PM	Developing a National Nassau Grouper Conservation Plan	Focused Group Discussion (Moderator: Janeen Bullard) & Facilitators
2:50-3:00 PM	Conclusion	Dr. Craig Dahlgren, Research Scientist

Appendix II: Understanding and managing fish populations: keeping the toolbox fit for purpose

Manuscript published in Journal of Fish Biology

Authors: Paris JR, Sherman KD, Bell E, Boulenger C, Delord, C, El-Mahdi MBM, Fairfield EA, Griffiths AM, Gutmann Roberts, C, Hedger RD, Holman LE, Hooper LH, Humphries NE, Katsiadaki I, King RA, Lemopoulos A, Payne CJ, Peirson G, Richter KK, Taylor MI, Trueman CN, Hayden B, Stevens JR

Journal of FISH BIOLOGY

fsbi An International Society for Fish Biology

Journal of Fish Biology (2018) **92**, 727–751
doi:10.1111/jfb.13549, available online at wileyonlinelibrary.com

Understanding and managing fish populations: keeping the toolbox fit for purpose

J. R. Paris*†##, K. D. Sherman*##, E. Bell‡, C. Boulenger§, C. Delord§, M. B. M. El-Mahdi‖, E. A. Fairfield‡, A. M. Griffiths*, C. Gutmann Roberts¶, R. D. Hedger**, L. E. Holman††, L. H. Hooper*, N. E. Humphries‡‡, I. Katsiadaki§§, R. A. King*, A. Lemopoulos‖‖, ¶¶, C. J. Payne***, G. Peirson†††, K. K. Richter‡‡‡, M. I. Taylor‡, C. N. Trueman††, B. Hayden§§§# and J. R. Stevens*‖‖‖‖#

**Biosciences, University of Exeter, Stocker Road, Exeter EX4 4QD, U.K., †School of Life Sciences, University of Sussex, Brighton, BN1 9QG, UK, ‡School of Biological Science, University of East Anglia, Norwich Research Park, Norwich, NR4 7UG, U.K., §ESE, Ecology and Ecosystem Health, Agrocampus Ouest, INRA, 35042 Rennes, France, ‖Faculty of Science, Laboratory of Molecular Genetics and Molecular Biology, Zoology Department, South Valley University, Qena 83523, Egypt, ¶Department of Life and Environmental Sciences, Faculty of Science and Technology, Bournemouth University, Bournemouth BH12 5BB, U.K., **Norwegian Institute for Nature Research, Trondheim, NO-7485, Norway, ††Ocean and Earth Science, National Oceanography Centre, University of Southampton, European Way, Southampton, SO14 3ZH, U.K., ‡‡Marine Biological Association of the United Kingdom, The Laboratory, Citadel Hill, Plymouth, PL1 2PB, UK, §§Cefas, Weymouth Laboratory, Weymouth, DT4 8UB, U.K., ‖‖Department of Environmental and Biological Sciences, University of Eastern Finland, P.O. Box 111, FI-80101, Joensuu, Finland, ¶¶Department of Biology, University of Turku, FI - 20014, Turku, Finland, ***Institute of Aquaculture, Faculty of Natural Sciences, University of Stirling, Stirling, FK9 4LA, U.K., †††Environment Agency, Research Analysis and Evaluation, Mance House, Kidderminster, DY11 7RA, U.K., ‡‡‡BioArCh, Department of Archaeology, University of York, York, YO10 5DD, U.K. and §§§Biology Department, Canadian Rivers Institute, University of New Brunswick, Fredericton, New Brunswick, E3B 5A3, Canada*

Wild fish populations are currently experiencing unprecedented pressures, which are projected to intensify in the coming decades. Developing a thorough understanding of the influences of both biotic and abiotic factors on fish populations is a salient issue in contemporary fish conservation and management. During the 50th Anniversary Symposium of The Fisheries Society of the British Isles at the University of Exeter, UK, in July 2017, scientists from diverse research backgrounds gathered to discuss key topics under the broad umbrella of 'Understanding Fish Populations'. Below, the output of one such discussion group is detailed, focusing on tools used to investigate natural fish populations. Five main groups of approaches were identified: tagging and telemetry; molecular tools; survey tools; statistical and modelling tools; tissue analyses. The appraisal covered current challenges and potential solutions for each of these topics. In addition, three key themes were identified as applicable across all tool-based applications. These included data management, public engagement, and fisheries policy and governance. The continued innovation of tools and capacity to integrate interdisciplinary approaches

‖‖‖‖Author to whom correspondence should be addressed. Tel.: +44 1392 723 775; email: j.r.stevens@exeter.ac.uk
##Joint first authors
#Joint senior authors

into the future assessment and management of fish populations is highlighted as an important focus for the next 50 years of fisheries research.

Key words: archaeology; genetics; modelling; stable isotopes; surveys; telemetry.

INTRODUCTION

Approximately 30% of fish species have been overexploited (FAO, 2014), representing significant losses to biodiversity, ecosystem services and socioeconomic contributions (Worm *et al.,* 2009). In light of the increasing challenges presented by climate change and other natural and anthropogenic stressors (Gordon *et al.,* 2018), an improved understanding of fish populations is critical to facilitate effective management and conservation initiatives. In July 2017, The Fisheries Society of the British Isles held its 50th Anniversary Symposium at the University of Exeter, UK, under the broad umbrella of 'Understanding Fish Populations'. To highlight key knowledge gaps and opportunities, we report the outcome of a working group convened at the symposium, which was tasked with considering the theme of tools for understanding fish populations. The scope of the discussion spanned diverse areas including spatial ecology and migration patterns, genetics and evolutionary biology, physiology, trophic ecology and developmental and population biology. In this article, we consider major advances in the use of tools across broad areas of fish biology and identify knowledge gaps and potential solutions in each area in order to guide and inform future research and to better understand and protect wild fish populations.

TAGGING AND TELEMETRY

A significant problem hampering the study of fish, marine benthic species in particular, is that of determining their geographical locations at fine scales, over long durations. Tagging and telemetry involves the application of external and or internal tags or devices to manually or passively track fish movement (Cooke *et al.,* 2013). Both forms can be particularly challenging in the marine environment, though manual tracking can work well at feeding grounds and at spawning aggregations (Murchie *et al.,* 2015), while passive tracking has valuable applications along known migration routes (Dahlgren *et al.,* 2016), for example, as anadromous and catadromous species migrate in and out of river estuaries (Lauridsen *et al.,* 2017). Suites of tools exist for such tasks [*e.g*. acoustic transmitters, passive information transponder (PIT) and Floy tags, radio, archival, *etc*.] and have been routinely used to understand the spatial ecology of a range of fish taxa (Bograd *et al.,* 2010). With technological improvements in tags and tracking equipment, the field has grown vastly in recent decades (Pine *et al.,* 2003; Jepsen *et al.,* 2015). We briefly highlight some of the tags and telemetry options commonly used by researchers along with a discussion of some of the limitations and challenges associated with these tools.

Archival data storage tags (DST), which can collect data on both the internal and external environments of fish are the only method available to assess internal states (*e.g*. bioenergetics; Cooke *et al.,* 2016). DSTs, however, currently only provide information on the environment experienced by the tagged fish if the tag is recovered, meaning these

data are lost if recapture rates are low, as is often the case in fish tagging surveys. Communication history acoustic tags (CHAT), which transmit data to nearby transponder receivers are a promising alternative. Since there have been relatively few uses of this tag type (Voegeli *et al.,* 2001; Hight & Lowe, 2007), there is potential for development in this area. Pop-off DSTs are also becoming available and may alleviate several of these concerns once problems associated with size and recoverability are resolved.

Pop-up Satellite Archival Tags (PSAT), which detach from the tagged fish after some time at sea and transmit telemetry data to overpassing satellites, are currently limited in terms of hardware, software and satellite reception. PSATs are large, so are limited in use for larger, often highly migratory individuals, and may also affect fish behaviour (Methling *et al.,* 2011). Additionally, battery failure, antenna damage, or mechanical failure may limit registration or transmission of data (Hays *et al.,* 2007; Musyl *et al.,* 2011). PSAT technology is relatively new, so future reductions in size and weight and also improvement in reliability can be expected. In terms of software, PSATs currently only transmit limited amounts of data due to transmission costs and the short time that the receiving satellite is above the horizon. Future software development is required to reduce transmission costs, optimise data transmission and provide more flexibility for users to tailor controls, in order to provide higher resolution data at the desired temporal scale. An increase in the number of satellite platforms that can receive PSAT data would help to improve reception issues. Interference on frequencies selected for tags at certain geographical locations (see Musyl *et al.,* 2011) also requires consideration.

Acoustic telemetry offers autonomous, continuous monitoring (Heupel *et al.,* 2006) and has the potential to significantly enhance our understanding of fish habitat use, activity patterns and resource partitioning (Hussey *et al.,* 2015). Acoustic arrays have been used in many studies elucidating fish movements (Papastamatiou *et al.,* 2013; Lea *et al.,* 2016) and transmitters have been used more innovatively to measure trophic interactions (Halfyard *et al.,* 2017). Issues remain however, in the significant cost and effort involved in deploying and maintaining acoustic arrays.

Organizations such as the Ocean Tracking Network (OTN; oceantrackingnetwork .org; Whoriskey, 2015) and the Integrated Marine Observing System (IMOS) animal tracking database Australian Animal Tracking Network (www.imos.org.au/facilities/animaltracking) both maintain acoustic infra-structure in the form of deployed receivers (arrays or curtains) in key ecological areas into which researchers are free to release tagged animals. These initiatives substantially reduce the cost and risk associated with acoustic tracking projects and similar approaches can be applied globally (*e.g*. a European tracking network is currently being developed). Furthermore, integration of standardised data repositories along with a comprehensive set of analytical tools to ensure rapid and sophisticated analysis of acoustic array data (Lea *et al.,* 2016) would lead to new insights into the spatial ecology of fish. Further technological developments such as the use of autonomous underwater vehicles (AUV) to perform routine data download operations, or even complement fixed acoustic receivers (Davis *et al.,* 2016), will make acoustic telemetry increasingly affordable and accessible to more researchers. Continued collaborations with established regional and international tracking networks, together with the ever-increasing sophistication, miniaturisation, durability and cost reduction of tags promises an increasingly important role for acoustic telemetry in our understanding of fish ecology.

MOLECULAR TOOLS

POPULATION GENETICS AND GENOMICS

Using molecular tools to understand fish genetic diversity and population structure has wide-ranging applications for evolutionary biology and the conservation and management of fish stocks. Until recently, molecular techniques such as mitochondrial sequencing and the analysis of microsatellite loci have been used most commonly to explore intra-specific variation in fish and many other organisms (Ferguson & Danzmann, 1998; Chistiakov *et al.,* 2006). More recently, however, the increased availability and cost efficiency of high-throughput sequencing, which is capable of producing millions of sequencing reads [*e.g*. RADseq (Davey & Blaxter, 2011) and RNAseq (Wang *et al.,* 2009)], has revolutionized the fields of population and conservation genetics (Allendorf *et al.,* 2010). It is, however, important to appreciate what extra information high-throughput sequencing data can provide, the biases involved in study design and data generation and also how its usage might be optimised. Here, we seek to identify knowledge gaps in the field of fish population genetics and contemplate how this area of research may evolve in the future.

Attaining high quality, clean DNA for large numbers of individuals is paramount for downstream sequencing processes, but in some cases can be challenging. Biological samples can often be compromised during sampling or transport, potentially rendering field efforts futile. Population genetic studies on fish frequently require sampling from river transects or remote locations at sea and so portable laboratories for sampling, storing and extracting DNA would be welcomed. Emerging technologies, *e.g*. the MinION USB sequencer (www.nanoporetech.com/products/minion), have the potential to revolutionize when and where genetic data can be generated. Most new technologies are currently restricted to sequencing small genomes, such as those of bacteria, but with on-going improvements, these technologies open up the possibility of being able to sequence DNA in real-time in the field (Hayden, 2015). Recently, the MinION technology has been used in hybrid assemblies with Illumina short reads (Austin *et al.,* 2017) and *de novo* eukaryotic genomes (including fish) are in progress (Jansen *et al.,* 2017).

Alongside population genetic studies, research based on whole genome data is emerging and the genomes of several commercially important species have now been published (*e.g*. Atlantic cod *Gadus morhua* L. 1758; Star *et al.,* 2011; Atlantic salmon *Salmo salar* L. 1758; Lien *et al.,* 2016). While the ever-reducing cost of whole genome sequencing provides opportunities to sequence and publish more fish genomes, in our view, the key priority is not simply publishing genomes, but also attaining high-quality genome annotation. Gene annotation and accurate knowledge of the function of different identified regions is of extreme importance if genomic tools are to be used reliably in conservation and management (Ekblom & Wolf, 2014). Therefore, projects such as the 'Functional Annotation of All Salmonid Genomes' (Macqueen *et al.,* 2017) should be encouraged and developed. It is also important not to underestimate or neglect the computing power and bioinformatics expertise required to produce high quality genome scaffolds and annotations, and also to recognise and account for biases in next generation sequencing data (Benestan *et al.,* 2017).

Furthermore, population genetic approaches are usually focused on a single species. Consequently, there is a mismatch between studies of a single species genotyped at high resolution, but generally at small spatial scales (*e.g*. population genetics, often

using hundreds to thousands of markers through genotyping by sequencing (GBS) or genome-wide association studies (GWAS)), and studies of multiple species at larger spatial scales but using lower resolution markers [*e.g*. phylogeography or biodiversity assessments using metabarcoding or mitochondrial (mt)DNA sequencing]. Nonetheless, the widespread application of molecular resources has led to the accumulation of rich datasets across a broad range of species, geographical regions and time periods (Blanchet *et al.,* 2017). Accordingly, we anticipate that this aggregation of data may allow the underlying processes that drive genetic variability across these regions and times to be revealed, enabling a broader testing of theories in population genetics and evolution (Pauls *et al.,* 2014; Ellegren & Galtier, 2016).

Such studies will require the combination of high genetic resolution markers across large spatial scales, which is a non-trivial task, especially when dealing with non-model species. Three challenges arise in such cases: firstly, the financial investment required to obtain reliable datasets for several species remains significant. Despite reductions in sequencing costs, it may be financially sensible to rely on more classical markers such as microsatellites or small subsets of single nucleotide polymorphisms (SNPs). Secondly, there is a need for a standardised framework in order to make datasets comparable across different species and regions. This standardization must occur when collecting samples, characterising markers (Ellis *et al.,* 2011; Helyar *et al.,* 2011) and during the subsequent data analysis to streamline user choices (Paris *et al.,* 2017), which may bias the biological interpretation of data (Rodríguez-Ezpeleta *et al.,* 2016). It is therefore important that researchers use common methods to isolate and characterise markers for entire sets of focal species and provide full access to detailed analyses when datasets are generated.

Finally, as multi-species approaches remain scarce, there is a need to define hypotheses at the beginning of such investigations. In this respect, simulation tools (Laval & Excoffier, 2004; Peng & Kimmal, 2005; Neuenschwander, 2006) are particularly useful for testing complex hypotheses and also for predictive purposes. Moreover, the integration of mathematical and statistical models with fish population genetics would be useful for revealing genotype–phenotype interactions (Ritchie *et al.,* 2015), evolutionary signatures (Stark *et al.,* 2007), functional DNA elements (Schrider & Kern, 2014), spatial dynamics (Guillot *et al.,* 2009) and species-genetic diversity correlations (SGDC; Vellend, 2003; Vellend *et al.,* 2014).

ENVIRONMENTAL DNA

The use of environmental (e)DNA to identify the presence and understand the distribution of fish has expanded rapidly in the past decade. eDNA is a polydisperse mixture (Turner *et al.,* 2014; Wilcox *et al.,* 2015) of various biological material ranging from entire cellular fragments to extracellular DNA, which is isolated from environmental samples such as water or sediment. Such techniques are used for species identification and food security purposes. Universal primers that target mtDNA can be applied for identifying species presence (Yamamoto *et al.,* 2016) or to gain information about species interactions (*e.g*. food-web construction; Sousa *et al.,* 2016).

An important component of this work is validating the results from eDNA surveys with traditional fish survey methods. In both freshwater and marine environments, eDNA has compared favourably with traditional fish survey methods (Thomsen *et al.,* 2012; Hänfling *et al.,* 2016). eDNA, however, was found to be less effective compared

with experienced snorkel surveys (Ulibarri *et al.,* 2017). This underpins the importance of validation with traditional techniques, especially in spatially heterogeneous and complex aquatic environments (Shogren *et al.,* 2017).

The development of effective PCR primers is central to the successful application of eDNA (Freeland, 2016; MacDonald & Sarre, 2017). As a result, a vast range of primer sets are available for fishes (Doi *et al.,* 2015; Clusa *et al.,* 2017). Metabarcoding primers, that simultaneously amplify eDNA from many fish species, have also been developed for monitoring entire fish communities (Miya *et al.,* 2015; Valentini *et al.,* 2016).

Beyond inferring if a fish species is present in the sampled location, researchers have begun to investigate if eDNA can provide further information regarding fish populations. The use of eDNA to infer population-level variation has been demonstrated (Uchii *et al.,* 2016; Sigsgaard *et al.,* 2016), but is still in its infancy. Similarly, although attempts to link eDNA concentration and fish biomass have shown promising results (Lacoursière-Roussel *et al.,* 2016; Yamamoto *et al.,* 2016), further development is required to improve the accuracy of these measurements. However, for applications utilising eDNA to be optimised, preexisting molecular information needs to be accessible. A number of publicly available databases [*e.g.* NCBI Genbank (www.ncbi.nlm.nih.gov/genbank/) and BOLD (www.boldsystems.org)] hold a vast array of molecular data, but there is still a need for further mitochondrial genome sequencing to allow for optimal usage of molecular identification techniques.

MICROBIOMES

Analysis of a microbiome can provide novel insights into the health and biology of fish populations. Traditional culture-dependent tools used to map the commensal microbiota community in fish are often time-consuming, expensive and subjected to bias as only 0·1–10% of bacteria can be cultured *in vitro* (Amann *et al.,* 1995; Austin, 2006). More recently, rapid culture-independent tools such as 16S ribosomal (r)RNA targeted sequencing have been used to provide detailed profiles of the structure and diversity of the microbiota residing on the mucosal surface of fish (Ghanbari *et al.,* 2015).

The gut microbiome composition has also become an important biomarker for understanding the influence of stress in fish (Llewellyn *et al.,* 2014), as numerous stressful stimuli have been shown to alter the microbiome composition (Xia *et al.,* 2014; Gaulke *et al.,* 2016). The gut microbiome composition can provide insights into the ecology and physiology of fish in a range of areas such as ecological speciation (Sevellec *et al.,* 2014), the biology of migratory fish (Llewellyn *et al.,* 2016), trophic interactions within ecosystems (Ingerslev *et al.,* 2014) and adaptation to extreme environments (Song *et al.,* 2016).

There are a number of challenges currently facing fish microbiome research. At present, the majority of data regarding the microbiome composition in wild teleost fish originates from laboratory models (Tarnecki *et al.,* 2017). More studies are required to see if captive-reared animals provide a reliable analogue for wild populations. Standardised protocols for collecting and generating microbiome data are also lacking, which could restrict progress as several processes have the potential to introduce differential bias in microbiota profiles (Salipante *et al.,* 2014; Hart *et al.,* 2015). Adopting a framework of robust, quality-controlled protocols (*e.g.* similar to human microbiome

research; Methé *et al.,* 2012) would be of great benefit. In addition, there is currently a lack of non-invasive protocols for conducting longitudinal or repeated sampling of the gut microbial community in individual fish over time. The application of rectal swabs (Budding *et al.,* 2014) for sampling the vent of fish could provide a non-invasive strategy for collecting such data. Finally, time-series data could also enhance our knowledge in terms of the functional aspects of host lifecycles and the stability and resilience of microbiota (Goodrich *et al.,* 2014).

SURVEY TOOLS

FIELD-BASED SURVEYS

Fish population assessments are conducted using a wide range of techniques; the advantages, limitations, personnel requirements and health and safety considerations of each are presented in Table I. It is encouraging to note that even well-established methods such as hydroacoustics are continually being improved, while emerging tools such as eDNA are beginning to be included in routine monitoring. We suggest that integrating methods and data series are key priorities for future research in this field.

In large and complex habitats, it is often the case that a suite of survey methodologies has to be employed to sample different times, habitats and species effectively. Indeed, an advantage of field-based surveys is the ability to generate information from both fishery-independent (Nash *et al.,* 2016) and fishery-dependent (Shin *et al.,* 2010) data. The availability of a diversity of methodologies, however, can make the task of assessment in these habitats even more costly; issues also remain over how to use often disparate data types to develop a sound understanding of a fishery. Integrating methods represents a key means of improving data resolution from field surveys. For instance, methods such as eDNA and hydroacoustic sampling provide comparatively fast and non-invasive estimates of fish community structure and biomass. To obtain a thorough understanding of fish populations, however, this information must be combined with fish age, size and health data, typically obtained *via* destructive sampling (*e.g*. gill netting). As yet, there are no structured, universally agreed guidelines on which methods should be integrated to obtain a thorough assessment of population dynamics from a specific habitat type.

Fish-survey methodologies are typically determined at a national level, making international comparisons of data extremely challenging. In recent years, standardised protocols initiated through the European Union Water Framework Directive (E.C., 2000) have facilitated Europe-wide assessments of fish community structure. Such international standardisation is essential when assessing anthropogenic effects on fish (Gordon *et al.,* 2018) and we recommend that efforts are made to make national datasets available using standardised metadata and biodiversity information, ideally *via* open sharing platforms (*e.g*. www.freshwaterplatform.eu).

HISTORICAL RECORDS

Historical records (*e.g*. catch records) can also be useful in helping to extrapolate population data back into the recent past. Libraries and historical societies often hold picture archives and these images can in some instances be used as a form of historical

TABLE I. Summary of popular current and emerging methods used for fish surveys along with the associated advantages and limitations of each method

Method	Advantages	Limitations	Personnel requirement	Health & safety consideration
Electric fishing	Can be used in flowing and still water, among macrophytes and obstructions; relatively unselective; can be used quantitatively	Inefficient in water >1–1·5 m depth or in wide reaches; limited by water and bed conductivity; can be harmful to sensitive fish species and life stages; invasive	Significant to high	High
Seine netting	Can be used quantitatively; efficiency well-understood; relatively unselective	Limited effectiveness in very deep or very shallow water; limited effectiveness where there are macrophytes, obstructions, or soft sediment; restricted to use in low velocity water bodies; invasive	High	Significant
Trawling	Large areas of deep water can be surveyed efficiently	Restricted to use in relatively open continuous stretches of water of >2 m depth; cannot be used where there are dense growths of macrophytes, very variable bed profiles or large debris; requires sizeable boats and launching facilities; invasive	High	High
Gill netting	Can be used in a wide variety of environments among debris and macrophytes, in almost any depth	Invasive–destructive; limited ability to assess absolute fish abundance	Significant	Significant
Hydroacoustics	Huge expanses of water can be surveyed efficiently; non-invasive; quantitative abundance estimates possible	Limited effectiveness in turbulent environments; can only sample relatively open water so unsuitable to use for sampling in marginal habitats; lacks capacity to differentiate between species; cannot assess age, condition and health of fish	Significant	Significant

TABLE I. Continued

Method	Advantages	Limitations	Personnel requirement	Health & safety consideration
Fyke netting and trapping	Can be deployed in a variety of Environments; can be effective for some species difficult to sample by other methods	Very species and size-selective; limited ability to assess absolute fish abundance	Significant	Significant
Fry surveys: micromesh seine, handnet & traps	Focuses on margins of rivers and lakes, therefore less resource intensive, simple equipment only; assesses a key life stage; relatively unselective	Only assesses juvenile populations; invasive as very young fish are unlikely to survive capture	Significant	Significant
Fish counters & fixed traps: sometimes accompanied by cameras (video recorder)	Good for assessing highly mobile fish with relatively predictable migration patterns	Resource intensive, high capital costs, maintenance; quantitative assessment for migratory species only; often only operational under certain environmental conditions	High	Significant
Rod-and-line	Adaptable, can be deployed almost anywhere; amenable to volunteers and citizen-science participation	Very effort-dependent (quantity and quality); strongly influenced by conditions; very selective for species and size of fish; limited capability to assess absolute fish abundance; very noisy data	High	Low
Commercial catch monitoring	Enables large volumes of data collected over large spatial and temporal scales; relatively inexpensive as fish are being caught anyway	Can only happen where commercial fisheries exist; little control over changes in effort and methodology, which are driven by market forces; strongly influenced by conditions	Low	Low
Visual surveys: snorkelling, diving, counting from the bank	Relatively non-invasive; enables observation of fish in their surroundings	Only applicable in high water clarity and over short ranges; mostly applicable to species with distinct individual home range, typically associated with physical habitat features	Moderate	Significant to high

Table I. Continued

Method	Advantages	Limitations	Personnel requirement	Health & safety consideration
Methods under development				
eDNA: single-target and meta barcoding	Very adaptable, deployable anywhere; non-invasive; non-selective; low field manpower requirement	Currently can only establish fish presence and abundance of species relative to each other, absolute abundance remains a challenge; cannot assess age, size, condition or health; uncertainty around the source of eDNA in lotic environments; high laboratory time requirement	Significant	Significant
DIDSON*–ARIS#high resolution sonar	Can be used in turbid water, among obstructions; can be used in a variety of depths and flows except very turbulent water; enables visualization of target fish, species identification; quantitative estimates possible; species (some) and size of fish can be identified; observations of fish behaviour possible; non-invasive	Mobile deployment currently challenging; limited ability to assess whole water-body abundance; limited species identification capability; high data-processing requirement; cannot assess age, condition and health of fish	Significant	Significant

*Dual-frequency IDentification *SONar*.

#Adaptive Resolution Imaging Sonar.

survey data to provide information on past community composition and size distributions (McClenachan, 2009). Historical records of catch data are typically held by government agencies or can be found in local archives (*e.g.* angling club logs) and corporate records. Such data have been used successfully to reconstruct fish populations back to the late 1800s (Thurstan *et al.,* 2010; Thurstan & Roberts, 2010). Catch reconstruction approaches can also provide useful insights into fishery trends that may not be apparent from data reported only by the U.N. Food and Agriculture Organization (FAO, 2015; Smith & Zeller, 2015; Zeller *et al.,* 2015). Although limited to the information that is still available, and subject to the often-unidentifiable biases of the individuals who originally recorded the data, this can provide a unique way to extrapolate population data back in time.

STATISTICAL AND MODELLING TOOLS

BAYESIAN METHODS

Reliable estimates of demographic variables (*e.g.* abundance, survival, growth rates and fecundity) and an understanding of the processes that regulate these variables are fundamental for sustainable management of fish populations. To understand the ecological processes in order to truly inform policy, however, researchers must use multiple data sources, provide links between management actions and population responses, and also estimate uncertainty as a prerequisite to making forecasts that provide useful information. Bayesian methods in ecology and conservation biology are now increasingly being used to explore these links, for example, in stable-isotope analyses. Indeed, the Bayesian framework provides an intuitive method for estimating parameters, expressing uncertainty in these estimates and allows for the incorporation of as much or as little existing data or prior knowledge that is available (Ellison, 2004). To develop the use of this specific framework in fish ecology and management, however, there is a need to educate and train fish biologists in the use of Bayesian principles and methods.

INDIVIDUAL-BASED MODELS

Individual-based models (IBM) are process-based mechanistic computer models that simulate emergent properties of fish biology, behaviour, traits or group characteristics, based on simple heuristic functions. The use of IBMs in fish research has grown exponentially (DeAngelis & Mooij, 2005) as computational power has increased (DeAngelis & Grimm, 2014). Several IBMs were presented at the 50th Anniversary Symposium of The Fisheries Society of the British Isles, and with continued increases in computational power, IBMs look set to offer powerful new avenues for population research (DeAngelis & Grimm, 2014) in computationally challenging multifactor systems such as fish ecotoxicology (Mintram *et al.,* 2017). Additionally, a variety of tools now exist which provide for the easier creation of new models, such as various R packages (www.r-project.org) and programmable environments (*e.g.* NetLogo; www.ccl.northwestern.edu/netlogo). Programmes such as R, however, are sometimes not intuitive to new users and so additional training for fisheries scientists and collaborations between scientists from different computational and statistical backgrounds would be advantageous. For a more robust future application of IBMs within fisheries science, there

is a need for further assessment of the relative strengths and weaknesses (and potential availability and future development) of the different models.

Integration with environmental data is a pertinent issue when modelling and is becoming easier through developments in geographic information systems (GIS) and other programming environments (such as R), which now include procedures and libraries for use in ecological work. One example is the use of food-web models that integrate environmental data (Christensen & Walters, 2004) and coral-reef ecosystem modelling methods (Rogers *et al.,* 2014; Weijerman *et al.,* 2015). A hindrance to the integration of environmental data into fisheries science is that it can be difficult to find and access data sources, although availability and accessibility of such data is improving (www.worldclim.org). The existence of a central node or hub with paths to these data sources would be useful.

TISSUE ANALYSIS

STABLE-ISOTOPE ECOLOGY

Stable isotopes are now routinely used to quantify the trophic ecology *(*Boecklen *et al.,* 2011) and migration history (Trueman *et al.,* 2012) of fish, or to identify community level patterns in food-web structure and resource use (Layman *et al.,* 2012). Although the technique is still in its relative infancy, stable-isotope ecology (or stable-isotopes analysis, SIA) has advanced much in recent decades. Below we outline four rapidly developing areas with the potential to enhance the applicability of this tool to studies of fish biology.

BIOCHEMICAL MECHANISM

The relationship between the isotopic composition of a consumer's tissues and that of its prey is fundamental to all applications of stable isotopes in ecology. While general principles are clear [*i.e*. faster reaction rates and preferential incorporation of light isotopes into excretory metabolites a process termed trophic fractionation (DeNiro & Epstein, 1977)], the precise mechanisms leading to fractionation and, particularly, the extent of isotopic fractionation expected under differing physiological conditions cannot currently be predicted, primarily due to the complexity of amino-acid biochemistry. Uncertainties associated with the isotopic expression of tissue composition and relative rates of tissue growth and regeneration further complicate the interpretation of stable isotope values in ecology. Recent information gained from compound-specific isotope analysis (*i.e*. assessing isotopic compositions of single amino acids), however, is beginning to shed light on the fractionation process (McMahon & McCarthy, 2016).

POPULATION-LEVEL DATA

The distribution of isotopic compositions of individuals within a population (often termed the isotopic niche; Newsome *et al.,* 2007) has been proposed as a powerful comparative measure of population-level ecological characters. In addition to individual variability in consumers, however, the distribution of isotopic compositions in a population is influenced by spatial and temporal variations in the isotopic composition of primary production, temporal variability within trophic linkages and differential

rates of growth and isotopic assimilation (Gorokhova, 2017). Very few studies have attempted to combine ecological and food web theory with isotope systematics to explore the sensitivity of community isotopic metrics to changes in food web structure and function.

ISOBANK

To date, applications of stable isotopes to fish biology have predominantly focussed on analyses of specific populations or communities. The absence of a centralised, open-access repository for stable-isotope data restricts the opportunity for syntheses or meta-analyses of stable-isotope data (Pauli *et al.,* 2017). Recent efforts to address this have found broad support from the stable isotope research community (Pauli *et al.,* 2017) and would be especially beneficial to fish biologists due to the large amount of fish-isotope data currently available. Defining an ontology of stable-isotope metadata, information required to describe and interpret isotope data, for fish biologists is an immediate requirement in this regard.

MARINE ISOSCAPES

The stable-isotope ratios of a consumer's tissue encode the resources (water, air, prey *etc*.) it was using when that tissue was formed. As such, provided one has access to a suite of isotopic baseline measurements (*e.g*. water, plants and primary consumers), it is possible to trace an organisms route through space and time up to the point of capture (Trueman *et al.,* 2012). Creation of a practically useful isoscape requires relatively dense sampling of a reference organism across space (and potentially time). Bulk stable-isotope analyses are now routine, commonly available globally and relatively cheap and regional marine-isoscape models are being developed at a rapid rate (MacKenzie *et al.,* 2014; Kurle & McWhorter, 2017). In the open ocean, sample-based isoscapes are difficult to develop, but progress is being made in isotope-enabled global biogeochemical models (Magozzi *et al.,* 2017), offering temporal and spatial models of expected isotopic variability at global scales. Improving the precision, accuracy and availability of these baseline measurements will increase the robustness and precision of isotope-based estimates animal position.

ARCHAEOLOGICAL MATERIAL

Archaeological material can allow an otherwise impossible snapshot into past populations. Traditional morphological approaches can provide age distributions and species ranges, and, with the rapid development of biomolecular archaeology in the past 20 years, many of the techniques used to explore modern fish populations can now be used to look into the past. From ancient DNA to proteomics and isotopes to lipids, a wide range of biomolecules have been recovered and explored from archaeological material (Orton, 2016). For example, compound-specific isotope analysis has the potential to track trophic level changes through time (McClelland & Montoya, 2002; Naito *et al.,* 2016). Population genetics of extinct populations have been successfully explored in terrestrial animals (Chang & Shapiro, 2016; Murray *et al.,* 2017) and these same techniques can be used on fish bones to reconstruct past genetics (Iwamoto *et al.,* 2012; Ólafsdóttir *et al.,* 2014). Ideally these data will be used to understand

environmental and anthropogenic effects on fish populations, and importantly, how modern fish populations might respond to climate change and fishing pressures.

A major barrier to the use of archaeological fish material is the fact that less than 10% of fish bones are identified to species level (Wheeler & Jones, 1989; Gobalet, 2001) and much of what is identified is buried in the grey literature of archaeological reports that are often printed in small quantities and not digitised (Linden & Webley, 2012). This makes the material relevant to an ecological question very difficult to find. Archaeologists are working towards ways to improve the amount of bones identified by better reference collections and education on fish bones (National Zooarchaeological Reference Resource, Nottingham's Archaeological Fish Resource; Vertebra@UWF) and on creating searchable databases of archaeological material (Callou, 2009; Kansa, 2010). In addition, new ZooMS (Zooarchaeology by mass spectrometry) techniques are being explored to quickly identify even small bones and scales to species using peptide mass fingerprinting (Richter *et al.,* 2011), which will allow even more material to be identified in a useful way for those working on understanding fish populations. In the near future, it should be possible for modern fish biologists, in conjunction with archaeologists, to ask direct questions of past populations (Van Neer & Ervynck, 2010).

GENERAL TOPICS IDENTIFIED AS APPLICABLE ACROSS ALL THEMES

MANAGEMENT OF DATA: INTEGRATION, CALIBRATION AND STANDARDIZATION

An integrated management framework for data classification, characterisation, storage and accessibility would be a valuable resource for fish and fisheries biologists. FishBase, which at the time of writing contains information regarding 33 600 fishes, involving 2290 collaborators, receiving over 600 000 visits per month, is an example of the potential for such a resource (www.fishbase.org; Froese & Pauly, 2017). A single database for all types of fish data (for example, DNA, tagging, isotopes, diet) is probably unworkable, but the advent of application programming interfaces (API) and analytical software, which allows automated querying across multiple databases, represents an unprecedented opportunity to access a wealth of global data. Indeed, we suggest that more data (such as those discussed here) could be integrated into FishBase. Such resources, however, require significant funding and long-term commitment from governments and trans-national organizations, *e.g.* the North Atlantic Salmon Conservation Organization (NASCO).

PUBLIC ENGAGEMENT, EDUCATION AND OUTREACH

Scientific engagement with the public is essential to effect meaningful societal change or to ensure a wider consensus is made around new discoveries or ethical considerations. Additionally, however, the power of the public as a tool in science is also being increasingly recognised. Crowdfunding, whereby a scientist requests small amounts of money from a large number of interested individuals to successfully launch a project, potentially provides a powerful new way to raise funds, overcoming some of the difficulties of raising money from traditional grant bodies, especially for early career researchers or those in developing countries (Wheat *et al.,* 2013).

In addition to funding science, the public can also actively engage in the process of research directly through citizen science projects. Whilst research conducted by non-professionals is certainly not a new concept, the numbers of projects involving citizen scientists are growing, especially in the fields of environmental science and ecology (Silvertown, 2009). Through catch records of amateur anglers and commercial net-fishery data extending back many years, research into fish and fisheries is uniquely placed to benefit from citizen science projects (Stuart-Smith *et al.,* 2013), which have effectively spanned generations of contributors. Similarly, REEF (www.reef.org) has been collecting reef-fish diversity and abundance data from trained volunteer divers for 27 years and the data have been successfully leveraged in hundreds of publications (Stallings, 2009; Serafy *et al.,* 2015). Citizen science can also help achieve important social outcomes, *e.g*. in establishing sustainable fisheries and marine protected areas (MPA) (Bonney *et al.,* 2014). As with crowdfunding, the best examples of citizen science typically encourage deeper engagement with the public and offer a pathway to the democratization of science.

FISHERIES POLICY AND GOVERNANCE

Conserving critical habitats is central to the sustainable management of fish species and populations. MPAs, networks of MPAs and marine conservation zones (MCZ) are widely accepted management tools for fish and other marine organisms that have been established in many countries (Harborne *et al.,* 2008; OSPAR Convention, 2013). The design of MPA networks could, however, benefit greatly from the integration of traditional survey data, along with modelling and connectivity data (Botsford *et al.,* 2009; *Gruss et al*., 2014). From a social science perspective, there is a need to better understand public perceptions of marine-related conservation issues, *e.g*. fishery regulations, MPAs and MCZs and to incorporate these data into fisheries policy and governance frameworks. For example, there is high public support for MPAs, with surveys showing that people desire around 40% of the UK's marine waters to be protected (Hawkins *et al.,* 2016). But, while the public appears to realise that levels of coverage are well below 40%, there is still a substantial disconnect between perceived coverage of highly protected UK MPAs (11%) and actual MPA coverage (<0·1%); ultimately, this means that people believe the UK oceans receive a higher level of conservation than in reality (Hawkins *et al.,* 2016). Developing and implementing effective policies for fisheries management remains challenging because of the complexities of fisheries and the socio-political landscape under which they typically operate (Jentoft & Chuenpagdee, 2009). The establishment of guidelines or frameworks for fisheries policy and governance (FAO, 2015), however, have the potential to better address these challenges and provide appropriate implementable solutions.

CONCLUSIONS

Across all five of the research themes identified here, it is clear that innovative and novel tools are being employed to understand all aspects of the biology of fish populations. Notwithstanding, the authors call for the continued development of these new and emerging techniques. In particular, there is a need for better integration of these methods and resulting data, to inform scientifically sound management and conservation

of fish populations. It should be noted, however, that not infrequently, revolutionary methods have been promoted as providing the ability to offer unprecedented novel answers to long-standing practical problems. Unfortunately, the danger is that such methods can (by their novelty and the excitement surrounding them), blinker scientists into posing questions that showcase the methodology, rather than the biology [*e.g.* the plethora of papers that emerged in the early 1990s extolling the virtues of the random amplified polymorphic DNA (RAPD) technique]. The potentially reduced power of using any technique on its own (new or otherwise), in isolation of other apparently antiquated methods can turn out to be unnecessarily restrictive. Every technique has its limitations, but often the restrictions of one tool can be substantially alleviated by the inclusion of another approach (Goodwin *et al.,* 2016; Nielsen *et al.,* 2017), the marriage of which can provide a new angle for researching challenging biological problems. It is important that both traditional and emerging tools remain in the toolbox of fish biology research.

Likewise, when genetic-based assignment became popular, many researchers naively believed the days of tagging fish were over. It is now realised that due to the many stochastic drivers of population structure, genetic stock identification-based methodologies such as genetic assignment, do not always succeed. In such cases, there remains a significant role for tagging in fish and fisheries research. As tag sizes decrease and the deleterious effects of tag insertions on fish also decrease, we can anticipate that genetics and tagging will both continue to have a role to play. The importance of the relative roles of each technique will depend on the questions being addressed, the population structure of the study species and the scale of the questions being assessed.

A final example, which highlights the importance of applying inter-disciplinary and complimentary tools for understanding fish populations, was a five-year, multi-agency, E.U. funded project investigating the migration and distribution of *S salar* in the north-east Atlantic (the SALSEA project; NASCO, 2008). The purpose was to understand not just where *S. salar* go, but what they eat, migration routes to feeding grounds and which waters and regions they pass through. The SALSEA project used a combination of genetics (microsatellites), SIA, at-sea trawls, tagging and gut contents analysis to assess the movements and diet of this species across the north-east Atlantic Ocean. As a result of applying these combined approaches, *S. salar* post-smolt movements have been confidently ascertained (Gilbey *et al.,* 2017). Nonetheless, even while this comprehensive study was being finalised, a similarly broad-ranging study was also being undertaken using SNPs (Bourret *et al.,* 2013). Arguably, this method offers both the potential for finer levels of stock discrimination and the ability to better explore patterns among functional loci, which may make microsatellite-based analysis redundant within a short period of time (although see Narum *et al.,* 2008).

Thus, the authors consider the continued development of emerging tools, together with the use of multiple methodologies and inter-disciplinary approaches, to represent the best avenues for further improving our understanding of fish populations. We implore scientists from unrelated fields to collaborate on such projects. The 50th Anniversary Symposium of The Fisheries Society of the British Isles represented one such event, where fish-focused researchers across diverse fields, came together to advance the state of fish biology.

We thank the organizing committee of the 50th Anniversary Symposium of The Fisheries Society of the British Isles for enabling the working group discussion that led to the development

of this review. Thanks also to the University of Exeter for hosting the symposium and to the numerous sponsors for funding its success. J.R.S. acknowledges the support of the SAMARCH project, an E.U. funded France Channel–Manche England Interreg initiative.

References

Allendorf, F. W., Hohenlohe, P. A. & Luikart, G. (2010). Genomics and the future of conservation genetics. *Nature Reviews Genetics* **11,** 697–709. https://doi.org/10.1038/nrg2844

Amann, R. I., Ludwig, W. & Schleifer, K. H. (1995). Phylogenetic identification and *in situ* detection of individual microbial cells without cultivation. *Microbiological Reviews* **59,** 143–169.

Austin, B. (2006). The bacterial microflora of fish, revised. *The Scientific World Journal* **6,** 931–945. https://doi.org/10.1100/tsw.2006.181

Austin, C. B., Hua Tan, M., Harrisson, K. A., Peng Lee, Y., Croft, L. J., Sunnucks, P., Pavlova, A. & Ming Gan, H. (2017). *De novo* genome assembly and annotation of Australia's largest freshwater fish, the Murray cod (*Maccullochella peelii*) from Illumina and Nanopore sequencing reads. *Gigascience* **6**(8), 1–6. https://doi.org/10.1093/gigascience/gix063

Benestan, L., Moore, J.-S., Sutherland, B. J. G., Le Luyer, J., Maaroufi, H., Rougeux, C., Normandeau, E., RYcroft, N., Atema, J., Harris, L. N., Tallman, R. F., Greenwood, S. J., Clark, F. K. & Bernatchez, L. (2017). Sex matters in massive parallel sequencing: evidence for biases in genetic parameter estimation and investigation of sex determination systems. *Molecular Ecology* **26**(24), 6767–6783. https://doi.org/10.1111/mec.14217

Blanchet, S., Prunier, J. G. & De Kort, H. (2017). Time to go bigger: emerging patterns in macrogenetics. *Trends in Genetics* **33,** 579–580. https://doi.org/10.1016/j.tig.2017.06.007

Boecklen, W. J., Yarnes, C. T., Cook, B. A. & James, A. C. (2011). On the use of stable isotopes in trophic ecology. *Annual Review of Ecology, Evolution and Systematics* **42,** 411–440. https://doi.org/10.1146/annurev-ecolsys-102209-144726

Bograd, S. J., Block, B. A., Costa, D. P. & Godley, B. J. (2010). Biologging technologies: new tools for conservation. Introduction. *Endangered Species Research* **10,** 1–7. https://doi.org/10.3354/esr00269

Bonney, R., Shirk, J. L., Phillips, T. B., Wiggins, A., Ballard, H. L., Miller-Rushing, A. J. & Parrish, J. K. (2014). Next steps for citizen science. *Science* **343,** 1436–1437. https://doi.org/10.1126/science.1251554

Botsford, L. W., Brumbaugh, D. R., Grimes, C., Kellner, J. B., Largier, J., O'Farell, M. R., Ralston, S., Soulanile, E. & Wespestad, V. (2009). Connectivity, sustainability and yield: bridging the gap between conventional fisheries management and marine protected areas. *Reviews in Fish Biology Fisheries* **19,** 69–95. https://doi.org/10.1007/s11160-008-9092-z

Bourret, V., Kent, M. P., Primmer, C. R., Vasermägi, A., Karlsson, S., Hindar, K., McGinnity, P., Verspoor, E., Bernatches, L. & Lien, S. (2013). SNP-array reveals genome-wide patterns of geographical and potential adaptive divergence across the natural range of Atlantic salmon (*Salmo salar*). *Molecular Ecology* **22,** 532–551.

Budding, A. E., Grasman, M. E., Eck, A., Bogaards, J. A., Vandenbroucke-Grauls, C. M., van Bodegraven, A. A. & Savelkoul, P. H. (2014). Rectal swabs for analysis of the intestinal microbiota. *PLoS One* **9,** e101344. https://doi.org/10.1371/journal.pone.0101344

Chang, D. & Shapiro, B. (2016). Using ancient DNA and coalescent-based methods to infer extinction. *Biology Letters* **12,** 20150822.

Chistiakov, D. A., Hellemans, B. & Volckaert, F. A. M. (2006). Microsatellites and their genomic distribution, evolution, function and applications: a review with special reference to fish genetics. *Aquaculture* **255,** 1–29.

Christensen, V. & Walters, C. J. (2004). Ecopath with Ecosim: methods, capabilities and limitations. *Ecological Modelling* **172,** 109–139.

Clusa, L., Ardura, A., Fernández, S., Roca, A. A. & García-Vázquez, E. (2017). An extremely sensitive nested PCR-RFLP mitochondrial marker for detection and identification of salmonids in eDNA from water samples. *PeerJ* **5,** e3045.

Cooke, S. J., Midwood, J. D., Thiem, J. D., Klimley, P., Lucas, M. C., Thorstad, E. B., Eiler, J., Holbrook, C. & Ebner, B. C. (2013). Tracking animals in freshwater with electronic tags: past, present and future. *Animal Biotelemetry* **1,** 5.

Cooke, S. J., Brownscombe, J. W., Raby, G. D., Broell, F., Hinch, S. G., Clark, T. D. & Semmens, J. M. (2016). Remote bioenergetics measurements in wild fish: opportunities and challenges. *Comparative Biochemistry and Physiology A* **202,** 23–37.

Dahlgren, C. P., Buch, K., Rechisky, E. & Hixon, M. A. (2016). Multiyear tracking of Nassau grouper spawning migrations. *Marine and Coastal Fisheries: Dynamics, Management and Ecosystem Science* **8,** 522–535. https://doi.org/10.1080/19425120.2016.1223233

Davey, J. W. & Blaxter, M. L. (2011). RADSeq: next-generation population genetics. *Briefings in Functional Genomics* **9,** 416–423.

DeAngelis, D. L. & Grimm, V. (2014). Individual-based models in ecology after four decades. *F1000Prime Reports* **6,** 39. https://doi.org/10.12703/P6-39

DeAngelis, D. L. & Mooij, W. M. (2005). Individual-based modelling of ecological and evolutionary processes. *Annual Review of Ecology, Evolution and Systematics* **36,** 147–168.

DeNiro, M. J. & Epstein, S. (1977). Mechanism of carbon isotope fractionation associated with lipid synthesis. *Science* **197,** 261–263.

Doi, H., Uchii, K., Takahara, T., Matsuhashi, S., Yamanaka, H. & Minamoto, T. (2015). Use of droplet digital PCR for estimation of fish abundance and biomass in environmental DNA surveys. *PLoS One* **10,** e0122763.

E.C (2000). Directive 2000/60/EC, establishing a framework for community action in the field of water policy. *Official Journal of the European Communities* **327,** 1–71 Available at www.eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2000:327:0001:0072:EN:PDF.

Ekblom, R. & Wolf, J. B. W. (2014). A field guide to whole-genome sequencing, assembly and annotation. *Evolutionary Applications* **7,** 1026–1042.

Ellegren, H. & Galtier, N. (2016). Determinants of genetic diversity. *Nature Reviews Genetics* **17,** 422–433. https://doi.org/10.1038/nrg.2016.58

Ellis, J. S., Gilbey, J., Armstrong, A., Balstad, T., Cauwelier, E., Cherbonnel, C., Consuegra, S., Coughlan, J., Cross, T. F., Crozier, W., Dillane, E., Ensing, D., García de Leániz, C., García-Vázquez, E., Griffiths, A. M., Hindar, K., Hjorleifsdottir, S., Knox, D., Machado-Schiaffino, G., McGinnity, P., Meldrup, D., Nielsen, E. E., Olafsson, K., Primmer, C. R., Prodohl, P., Stradmeyer, L., Vähä, J. P., Verspoor, E., Wennevik, V. & Stevens, J. R. (2011). Microsatellite standardization and evaluation of genotyping error in a large multi-partner research programme for conservation of Atlantic salmon (*Salmo salar* L.). *Genetica* **139,** 353–367.

Ellison, A. M. (2004). Bayesian inference in ecology. *Ecology Letters* **7,** 509–520.

FAO (2014). The State of World Fisheries and Aquaculture Opportunities and Challenges *2014*. Rome: FAO Available at http://www.fao.org/3/a-i3720e.pdf

FAO (2015). *Voluntary Guidelines for Securing Sustainable Small-Scale Fisheries in the Context of Food Security and Poverty Eradiacation*. Rome: FAO Available at http://www.fao.org/fishery/ssf/guidelines/en

Ferguson, M. M. & Danzmann, R. G. (1998). Role of genetic markers in fisheries and aquaculture: useful tools or stamp collecting? *Canadian Journal of Fisheries and Aquatic Sciences* **55,** 1553–1563.

Freeland, J. R. (2016). The importance of molecular markers and primer design when characterizing biodiversity from environmental DNA. *Genome* **60,** 358–374.

Gaulke, C. A., Barton, C. L., Proffitt, S., Tanguay, R. L. & Sharpton, T. J. (2016). Triclosan exposure is associated with rapid restructuring of the microbiome in adult zebrafish. *PLoS One* **11,** e.0154632.

Ghanbari, M., Kneifel, W. & Domig, K. J. (2015). A new view of the fish gut microbiome: advances from next-generation sequencing. *Aquaculture* **448,** 464–475. https://doi.org/10.1016/j.aquaculture.2015.06.033

Gilbey, J., Coughlan, J., Wennevik, V., Prodöhl, P., Stevens, J. R., Garcia de Leaniz, C., Ensing, D., Cauwelier, E., Cherbonnel, C., Consuegra, S., Coulson, M. W., Cross, T. F., Crozier, W., Dillane, E., Ellis, J. S., García-Vázquez, E., Griffiths, A. M., Gudjonsson, S., Hindar, K., Karlsson, S., Knox, D., Machado-Schiaffino, G., Meldrup, D., Nielsen, E. E., Ólafsson, K., Primmer, C. R., Prusov, S., Stradmeyer, L., Vähä, J.-P., Veselov, A. J., Webster,

L. M. I., McGinnity, P. & Verspoor, E. (2017). A microsatellite baseline for genetic stock identification of European Atlantic salmon (*Salmo salar* L.). *ICES Journal of Marine Science*. https://doi.org/10.1093/icesjms/fsx184

Gobalet, K. W. (2001). A critique of faunal analysis; inconsistency among experts in blind tests. *Journal of Archaeological Science* **28,** 377e386.

Goodrich, J. K., Di Rienzi, S. C., Poole, A. C., Koren, O., Walters, W. A., Caporaso, J. G., Knight, R. & Ley, R. E. (2014). Conducting a microbiome study. *Cell* **158,** 250–262. https://doi.org/10.1016/j.cell.2014.06.037

Goodwin, J. C. A., King, R. A., Jones, J. I., Ibbotson, A. & Stevens, J. R. (2016). A small number of anadromous females drive reproduction in a brown trout (*Salmo trutta*) population in an English chalk stream. *Freshwater Biology* **61,** 1075–1089.

Gordon, T. A. C., Harding, H. R., Clever, F. K., Davidson, I. K., Davison, W., Montgomery, D. W., Nedelec, S. L., Weatherhead, R. C., Windsor, F. M., Armstrong, J. D., Bardonnet, A., Bergman, E., Britton, J. R., Côté, I. M., D'Agostino, D., Greenberg, L. A., Harborne, A. R., Kahilainen, K. K., Metcalfe, N. B., Mills, S. C., Milner, N. J., Mittermayer, F. H., Montorio, L., Prokkola, L. A., Rutterford, L. A., Salvanes, A. G. V., Simpson, S. D., Vainikka, A., Pinnegar, J. K. & Santos, E. M. (2018). Fishes in a changing world: learning from the past to promote sustainability of fish populations. *Journal of Fish Biology* **92,** 804–827. https://doi.org/10.1111/jfb.13546

Gorokhova, E. (2017). Individual growth as a non-dietary determinant of the isotopic niche metrics. *Methods in Ecology and Evolution*. https://doi.org/10.1111/2041-210X.12887

Gruss, A., Robinson, J., Heppell, S. S., Heppell, S. A. & Semmens, B. X. (2014). Conservation and fisheries effects of spawning aggregation marine protected areas: what we know, where we should go and what we need to get there. *ICES Journal of Marine Science* **71,** 1515–1534. https://doi.org/10.1093/icesjms/fsu038

Guillot, G., Leblois, R., Coulon, A. & Frantz, A. C. (2009). Statistical methods in spatial genetics. *Molecular Ecology* **18,** 4734–4756. https://doi.org/10.1111/j.1365-294X.2009.04410.x

Halfyard, E. A., Webber, D., Del Papa, J., Leadley, T., Kessel, S. T., Colborne, S. F. & Fisk, A. T. (2017). Evaluation of an acoustic telemetry transmitter designed to identify predation events. *Methods in Ecology and Evolution* **8,** 1063–1071. https://doi.org/10.1111/2041-210X.12726

Hänfling, B., Lawson Handley, L., Read, D. S., Hahn, C., Li, J., Nichols, P., Blackman, R. C., Oliver, A. & Winfield, I. J. (2016). Environmental DNA metabarcoding of lake fish communities reflects long-term data from established survey methods. *Molecular Ecology* **25,** 3101–3119.

Harborne, A. R., Mumby, P. J., Cappel, C. V., Dahlgren, C. P., Micheli, F., Holmes, K. E., Sanchirico, J. E., Broad, K., Elliot, I. A. & Brumbaugh, D. R. (2008). Reserve effects and natural variation in coral reef communities. *Journal of Applied Ecology* **45,** 1010–1018.

Hart, M. L., Meyer, A., Johnson, P. J. & Ericsson, A. C. (2015). Comparative evaluation of DNA extraction methods from feces of multiple host species for downstream next-generation sequencing. *PLoS One* **10,** e0143334. https://doi.org/10.1371/journal.pone.0143334

Hawkins, J. P., O'Leary, B. C., Bassett, N., Peters, H., Rakowski, S., Reeve, G. & Roberts, C. M. (2016). Public awareness and attitudes towards marine protection in the United Kingdom. *Marine Pollution Bulletin* **111,** 231–236.

Hayden, E. C. (2015). Pint-sized DNA sequencer impresses first users. *Nature* **521,** 15–16.

Hays, G. C., Bradshaw, C. J. A., James, M. C., Lovell, P. & Sims, D. W. (2007). Why do Argos satellite tags deployed on marine animals stop transmitting? *Journal of Experimental Marine Biology and Ecology* **349,** 52–60.

Helyar, S. J., Hemmer-Hansen, J., Bekkevold, D., Taylor, M. I., Ogden, R., Limborg, M. T., Cariani, A., Maes, G. E., Dopere, E., Carvalho, G. & Nielsen, E. E. (2011). Application of SNPs for population genetics of nonmodel organisms: new opportunities and challenges: analytical approaches. *Molecular Ecology Resources* **11,** 123–136.

Heupel, M. R., Semmens, J. M. & Hobday, A. J. (2006). Automated acoustic tracking of aquatic animals: scales, design and deployment of listening station arrays. *Marine and Freshwater Research* **57,** 1–13.

Hight, B. V. & Lowe, C. G. (2007). Elevated body temperatures of adult female leopard sharks, *Triakis semifasciata*, while aggregating in shallow nearshore embayments: evidence for

behavioral thermoregulation? *Journal of Experimental Marine Biology and Ecology* **352,** 114–128.
Hussey, N. E., Kessel, S. T., Aarestrup, K., Cooke, S. J., Cowley, P. D., Fisk, A. T., Harcourt, R. G., Holland, K. N., Iverson, S. J., Kocik, J. F., Flemming, J. E. M. & Whoriskey, F. G. (2015). Aquatic animal telemetry: a panoramic window into the underwater world. *Science* **348,** 1221–1255642. https://doi.org/10.1126/science.1255642
Ingerslev, H. C., von Gersdorff Jørgensen, L., Strube, M. L., Larsen, N., Dalsgaard, I., Boye, M. & Madsen, L. (2014). The development of the gut microbiota in rainbow trout (*Oncorhynchus mykiss*) is affected by first feeding and diet type. *Aquaculture* **424–425**, 24–34. https://doi.org/10.1016/j.aquaculture.2013.12.032
Iwamoto, E. M., Myers, J. M. & Gustafson, R. G. (2012). Resurrecting an extinct salmon evolutionary significant unit: archived scales, historical DNA and implication for restoration. *Molecular Ecology* **21,** 1567–1582.
Jansen, H. J., Liem, M., Jong-Raadsen, S. A., Dufour, S., Weltzien, F.-A., Swinkels, W., Koelewijn, A., Palstra, A. P., Pelster, B., Spaink, H. P., Van den Thillart, G. E., Dirks, R. P. & Henkel, C. V. (2017). Rapid de novo assembly of the European eel genome from nanopore sequencing reads. *Sci Rep* **7**, 101907. https://doi.org/10.1101/101907
Jentoft, S. & Chuenpagdee, R. (2009). Fisheries and coastal governance as a wicked problem. *Marine Policy* **33,** 553–560. https://doi.org/10.1016/j.marpol.2008.12.002
Jepsen, N., Thorstad, E. B., Havn, T. & Lucas, M. C. (2015). The use of external electronic tags on fish: an evaluation of tag retention and tagging effects. *Animal Biotelemetry* **3**, 1–23. https://doi.org/10.1186/s40317-015-0086-z
Kansa, E. C. (2010). Open context in context: cyberinfrastructure and distributed approaches to publish and preserve archaeological data. *The SAA Archaeological Record* **10,** 12–16.
Kurle, C. M. & McWhorter, J. (2017). Spatial and temporal variability within marine isoscaes: implications for interpreting stable isotope data from marine systems. *Marine Ecology Progress Series* **568,** 31–45. https://doi.org/10.3354/meps12045
Lacoursière-Roussel, A., Rosabal, M. & Bernatchez, L. (2016). Estimating fish abundance and biomass from eDNA concentrations: variability among capture methods and environmental conditions. *Molecular Ecology Resources* **16,** 1401–1414.
Lauridsen, R. B., Moore, A., Privitera, L., Gregory, S. D., Beaumont, W. R. C. & Kavanagh, A. J. (2017). Migration behaviour and loss rate of trout smolts in the transitional zone between freshwater and saltwater. In *Proceedings of the 2nd International Sea Trout Symposium Sea Trout: Science & Management* (Harris, G., ed), pp. 292–307. Leicester: Matador.
Laval, G. & Excoffier, L. (2004). SIMCOAL 2.0: a program to simulate genomic diversity over large recombining regions in a subdivided population with a complex history. *Bioinformatics* **20,** 2485–2487. https://doi.org/10.1093/bioinformatics/bth264
Layman, C. A., Araujo, M. S., Boucek, R., Hammerschlag-Peyer, C. M., Harrison, E., Jud, Z. R., Matich, P., Rosenblatt, A. E., Vaudo, J. J., Yeager, L. A., Post, D. M. & Bearhop, S. (2012). Applying stable isotopes to examine food-web structure: an overview of analytical tools. *Biological Reviews* **87,** 545–562. https://doi.org/10.1111/j.1469-185X.2011.00208.x
Lea, J. S. E., Humphries, N. E., von Brandis, R. G., Clarke, C. R. & Sims, D. W. (2016). Acoustic telemetry and network analysis reveal the space use of multiple reef predators and enhance marine protected area design. *Proceedings of the Royal Society B* **283**(1834), 20160717. https://doi.org/10.1098/rspb.2016.0717
Lien, S., Koop, B. F., Sandve, S. R., Miller, J. R., Matthew, P., Leong, J. S., Minkley, D. R., Zimin, A., Grammes, F., Grove, H., et al. (2016). The Atlantic salmon genome provides insights into rediploidization. *Nature* **533,** 200–205.
Linden, M. V. & Webley, L. (2012). Introduction: development-led archaeology in north-west Europe. In *Development-led Archaeology in North-West Europe* (Bradley, R., Haselgrove, C., Linden, M. V. & Webley, L., eds). Oxford: Oxbow Books.
Llewellyn, M. S., Boutin, S., Hoseinifar, S. H. & Derome, N. (2014). Teleost microbiomes: the state of the art in their characterization, manipulation and importance in aquaculture and fisheries. *Frontiers in Microbiology* **5**, 1–17. https://doi.org/10.3389/fmicb.2014.00207
Llewellyn, M. S., McGinnity, P., Dionne, M., Letourneau, J., Thonier, F., Carvalho, G. R., Creer, S. & Derome, N. (2016). The biogeography of the Atlantic salmon (*Salmo salar*) gut microbiome. *The ISME Journal* **10,** 1280–1284. https://doi.org/10.1038/ismej.2015.189

MacDonald, A. J. & Sarre, S. D. (2017). A framework for developing and validating taxon-specific primers for specimen identification from environmental DNA. *Molecular Ecology Resources* **17,** 708–720.

MacKenzie, K. M., Longmore, C., Preece, C., Lucas, C. H. & Trueman, C. N. (2014). Testing the long-term stability of marine isoscapes in shelf seas using jellyfish tissues. *Biogeochemistry* **121,** 441–454. https://doi.org/10.1007/s10533-014-0011-1

Macqueen, D. J., Primmer, C. R., Houston, R. D., Nowak, B. F., Bernatchez, L., Bergseth, S., Davidson, W. S., Gallardo-Escárate, C., Goldammer, T., Guiguen, Y., Iturra, P., Kijas, J. W., Koop, B. F., Lien, S., Martin, S. A. M., McGinnity, P., Montecino, M., Naish, K. A., Nichols, K. M., Ólafsson, K., Omholt, S. W., Palti, Y., Plastow, G. S., Rexroad, C. E. III, Rise, M. L., Ritchie, R. J., Sandve, S. R., Schulte, P. M., Tello, A., Vidal, R., Vik, J. O., Wargelius, A., Yáñez, J. M. & The FAASG onsortium (2017). Functional annotation of all salmonid genomes (FAASG): an international initiative supporting future salmonid research, conservation and aquaculture. *BMC Genomics* **18,** 1–9.

Magozzi, S., Yool, A., Van der Zanden, H. B., Wunder, M. B. & Trueman, C. N. (2017). Using ocean models to predict spatial and temporal variation in marine carbon isotopes. *Ecosphere* **8,** e01763. https://doi.org/10.1002/ecs2.1763

McClelland, J. W. & Montoya, J. P. (2002). Trophic relationships and the nitrogen isotopic composition of amino acids in plankton. *Ecology* **83,** 2173–2180.

McClenachan, L. (2009). Documenting loss of large trophy fish from the Florida keys with historical photographs. *Conservation Biology* **23,** 636–643.

McMahon, K. W. & McCarthy, M. D. (2016). Embracing variability in amino acid δ^{15}N fractionation: mechanisms, implications and applications for trophic tcology. *Ecosphere* **7,** e01511.

Methé, B. A., Nelson, K. E., Pop, M., Creasy, H. H., Giglio, M. G., Huttenhower, C., Gevers, D., Petrosino, J. F., Abubucker, S., Badger, J. H. & Chinwalla, A. T. (2012). A framework for human microbiome research. *Nature* **486,** 215–221. https://doi.org/10.1038/nature11209

Methling, C., Tudorache, C., Skov, P. V. & Steffensen, J. F. (2011). Pop up satellite tags impair swimming performance and energetics of the European eel (*Anguilla anguilla*). *PLoS One* **6,** e20797. https://doi.org/10.1371/journal.pone.0020797

Mintram, K. S., Brown, A. R., Maynard, S. K., Thorbek, P. & Tyler, C. R. (2017). Capturing ecology in modeling approaches applied to environmental risk assessment of endocrine active chemicals in fish. *Critical Reviews in Toxicology*. https://doi.org/10.1080/10408444.2017.1367756

Miya, M., Sato, Y., Fukunaga, T., Sado, T., Poulsen, J. Y., Sato, K., Minamoto, T., Yamamoto, S., Yamanaka, H., Araki, H. & Kondoh, M. (2015). MiFish, a set of universal PCR primers for metabarcoding environmental DNA from fishes: detection of more than 230 subtropical marine species. *Open Science* **2,** 150088.

Murchie, K. J., Shultz, A. D., Stein, J. A., Cooke, S. J., Lewis, J., Franklin, J., Vincent, G., Brooks, E. J., Claussen, J. E. & Philipp, D. P. (2015). Defining adult bonefish (*Albula vulpes*) movement corridors around Grand Bahama in the Bahamian Archipelago. *Environmental Biology of Fishes* **98,** 2203–2212. https://doi.org/10.1007/s10641-015-0422-4

Murray, G. G. R., Soares, A. E. R., Novak, B. J., Schaefer, N. K., Cahill, J. A., Baker, A. J. & Shapiro, B. (2017). Natural selection shaped the rise and fall of passenger pigeon genomic diversity. *Science* **358**(6365), 951–954. https://doi.org/10.1101/154294

Musyl, M. K., Domeier, M. L., Nasby-Lucase, N., Brill, R. W., McNaughton, L. M., Swimmer, J. Y., Lutcavage, M. S., Wilson, S. G., Galuardi, B. & Liddle, J. B. (2011). Performance of pop-up satellite archival tags. *Marine Ecology Progress Series* **433,** 1–28.

Naito, Y. I., Chikaraishi, Y., Drucker, D. G., Ohkouchi, N., Semal, P., Wißing, C. & Bocherens, H. (2016). Ecological niche of Neanderthals from spy cave revealed by nitrogen isotopes of individual amino acids in collagen. *Journal of Human Evolution* **93,** 82–90.

Narum, S. R., Banks, M., Beacham, T. D., Bellinger, M. R., Campbell, M. R., DeKoning, J., Elz, A., Guthrie, C. M. III, Kozfkay, C., Miller, K. M., Moran, P., Phillips, R., Seeb, L. W., Smith, C. T., Warheit, K., Young, S. F. & Garza, J. C. (2008). Differentiating salmon populations at broad and fine geographic scales with microsatellites and SNPs. *Molecular Ecology* **17,** 3464–3477.

Nash, K. L., Bijoux, J., Robinson, J., Wilson, S. K. & Graham, N. A. J. (2016). Harnessing fishery-independent indicators to aid management of data-poor fisheries: weighing habitat and fishing effects. *Ecosphere* **7,** e01362.

Neuenschwander, S. (2006). AQUASPLATCHE: a program to simulate genetic diversity in populations living in linear habitats. *Molecular Ecology Notes* **6,** 583–585.

Newsome, S. D., del Rio, C. M., Bearhop, S. & Philiips, D. L. (2007). A niche for isotopic ecology. *Frontiers in Ecology and the Environment* **5,** 429–436.

Nielsen, J. M., Clare, E. L., Hayden, B., Brett, M. T. & Kratina, P. (2017). Diet tracing in ecology: method comparison and selection. *Methods in Ecology and Evolution (Online)* . https://doi.org/10.1111/2041-210X.12869

Ólafsdóttir, G. Á., Westfall, K. M., Edvardsson, R. & Pálsson, S. (2014). Historical DNA reveals the demographic history of Atlantic cod (*Gadus morhua*) in medieval and early modern Iceland. *Proceedings of the Royal Society B* **281,** 20132976.

Orton, D. C. (2016). Archaeology as a tool for understanding past marine resource use and its impact. In *Perspectives on Oceans Past* (Schwerdtner Máñez, K. & Poulsen, B., eds), pp. 47–69. Dordrecht: Springer.

Papastamatiou, Y. P., Meyer, C. G., Carvalho, F., Dale, J. J., Hutchinson, M. R. & Holland, K. N. (2013). Telemetry and random-walk models reveal complex patterns of partial migration in a large marine predator. *Ecology* **94,** 2595–2606.

Paris, J. R., Stevens, J. R. & Catchen, J. M. (2017). Lost in parameter space: a road map for stacks. *Methods in Ecology and Evolution* **8,** 1360–1373. https://doi.org/10.1111/2041-210X.12775

Pauli, J. N., Newsome, S. D., Cook, J. A., Harrod, C., Steffan, S. A., Baker, C. J. O., Ben-David, M., Bloom, D., Bowen, G. J., Cerling, T. E., Cicero, C., Cook, C., Dohm, M., Dharampal, P. S., Graves, G., Gropp, R., Hobson, K. A., Jordan, C., MacFadden, B., Pilaar Birch, S., Poelen, J., Ratnasingham, S., Russell, L., Stricker, C. A., Uhen, M. D., Yarnes, C. T. & Hayden, B. (2017). Opinion: why we need a centralized repository for isotopic data. *Proceedings of the National Academy of Sciences* **114,** 2997–3001. https://doi.org/10.1073/pnas.1701742114

Pauls, S. U., Alp, M., Bálint, M., Bernabò, P., Čiampor, F., Čiamporová-Zaťovičová, Z., Finn, D. S., Kohout, J., Leese, F., Lencioni, V., Paz-Vinas, I. & Monaghan, M. T. (2014). Integrating molecular tools into freshwater ecology: developments and opportunities. *Freshwater Biology* **59,** 1559–1576. https://doi.org/10.1111/fwb.12381

Peng, B. & Kimmal, M. (2005). SimuPOP: a forward-time population genetics simulation environment. *Bioinformatics* **21,** 3686–3687.

Pine, W. E., Pollock, K. H., Hightower, J. E., Kwak, T. J. & Rice, J. A. (2003). A review of tagging methods for estimating fish population size and components of mortality. *Fisheries* **28,** 10–13. https://doi.org/10.1577/1548-8446(2003)28

Richter, K. K., Wilson, J., Jones, A. K. G., Buckley, M., van Doorn, N. & Collins, M. J. (2011). Fish 'n chips: ZooMS peptide mass fingerprinting in a 96 well plate format to identify fish bone fragments. *Journal of Archaeological Science* **38,** 1502–1510.

Ritchie, M. D., Holzinger, E. R., Li, R., Pendergrass, S. A. & Kim, D. (2015). Methods of integrating data to uncover genotype-phenotype interactions. *Nature Reviews Genetics* **16,** 85–97. https://doi.org/10.1038/nrg3868

Rodríguez-Ezpeleta, N., Bradbury, I. R., Mendibil, I., Álvarez, P., Cotano, U. & Irigoien, X. (2016). Population structure of Atlantic mackerel inferred from RAD-seq-derived SNP markers: effects of sequence clustering parameters and hierarchical SNP selection. *Molecular Ecology Resources* **16,** 991–1001. https://doi.org/10.1111/1755-0998.12518

Rogers, A., Blanchard, J. L. & Mumby, P. J. (2014). Vulnerability of coral reef fisheries to a loss of structural complexity. *Current Biology* **24,** 1000–1005.

Salipante, S. J., Kawashima, T., Rosenthal, C., Hoogestraat, D. R., Cummings, L. A., Sengupta, D. J., Harkins, T. T., Cookson, B. T. & Hoffman, N. G. (2014). Performance comparison of Illumina and ion torrent next-generation sequencing platforms for 16S rRNA-based bacterial community profiling. *Applied and Environmental Microbiology* **80,** 7583–7591. https://doi.org/10.1128/AEM.02206-14

Schrider, D. R. & Kern, A. D. (2014). Discovering functional DNA elements using population genomic information: a proof of concept using human mtDNA. *Genome Biology and Evolution* **6,** 1542–1548. https://doi.org/10.1093/gbe/evu116

Serafy, J. E., Shideler, G. S., Araújo, R. J. & Nagelkerken, I. (2015). Mangroves enhance reef fish abundance at the Caribbean regional scale. *PLoS One* **10,** e0142022. https://doi.org/10.1371/journal.pone.0142022

Sevellec, M., Pavey, S. A., Boutin, S., Filteau, M., Derome, N. & Bernatchez, L. (2014). Microbiome investigation in the ecological speciation context of lake whitefish (*Coregonus clupeaformis*) using next-generation sequencing. *Journal of Evolutionary Biology* **27,** 1029–1046. https://doi.org/10.1111/jeb.12374

Shin, Y.-J., Shannon, L. J., Bundy, A., Coll, M., Aydin, K., Bez, N., Blanchard, J. L., Borges, M. D. F., Diallo, I., Diaz, E., Heymans, J. J., Hill, L., Johannesen, E., Jouffre, D., Kifani, S., Labrosse, P., Link, J. S., Mackinson, S., Masski, H., Möllmann, C., Neira, S., Ojaveer, H., Abdallahi, K. O. M., Perry, I., Thiao, D., Yemane, D. & Cury, P. M. (2010). Using indicators for evaluating, comparing and communicating the ecological status of exploited marine ecosystems. 2. Setting the scene. *ICES Journal of Marine Science* **67,** 692–716.

Shogren, A. J., Tank, J. L., Andruszkiewicz, E., Olds, B., Mahon, A. R., Jerde, C. L. & Bolster, D. (2017). Controls on eDNA movement in streams: transport, retention and resuspension. *Scientific Reports* **7,** 5065. https://doi.org/10.1038/s41598-017-05223-1

Sigsgaard, E. E., Nielsen, I. B., Bach, S. S., Lorenzen, E. D., Robinson, D. P., Knudsen, S. W., Pedersen, M. W., Al Jaidah, M., Orlando, L., Willerslev, E. & Møller, P. R. (2016). Population characteristics of a large whale shark aggregation inferred from seawater environmental DNA. *Nature Ecology & Evolution* **1,** 0004.

Silvertown, J. (2009). A new dawn for citizen science. *Trends in Ecology & Evolution* **24,** 467–471. https://doi.org/10.1016/j.tree.2009.03.017

Smith, N. S. & Zeller, D. K. (2015) Unreported catch and tourist demand on local fisheries of small island states: these case of The Bahamas, 1950–2010. *Fishery Bulletin* **114,** 117–131. https://doi.org/10.7755/FB.114.1.10

Song, W., Li, L., Huang, H., Jiang, K., Zhang, F., Chen, X., Zhao, M. & Ma, L. (2016). The gut microbial community of antarctic fish detected by 16S rRNA gene sequence analysis. *BioMedical Research International*. https://doi.org/10.1155/2016/3241529

Sousa, L. L., Xavier, R., Costa, V., Humphries, N. E., Trueman, C., Rosa, R., Sims, D. W. & Queiroz, N. (2016). DNA barcoding identifies a cosmopolitan diet in the ocean sunfish. *Scientific Reports* **6,** 1–9.

Stallings, C. D. (2009). Fishery-independent data reveal negative effect of human population density on Caribbean predatory fish communities. *PLoS One* **4,** e5333. https://doi.org/10.1371/journal.pone.0005333

Star, B., Nederbragt, A. J., Jentoft, S., Grimholt, U., Malmstrom, M., Greger, T. F., Rounge, T. B., Paulsen, J., Solbakken, M. H., Sharma, A., Wetten, O. F., Lanzén, A., Winer, R., Knight, J., Vogel, J.-H., Aken, B., Andersen, Ø., Lagesen, K., Tooming-Klunderud, A., Edvardsen, R. B., Tina, K. G., Espelund, M., Nepal, C., Previti, C., Karlsen, B. O., Moum, T., Skage, M., Berg, P. R., Gjøen, T., Kuhl, H., Thorsen, J., Malde, K., Reinhardt, R., Du, L., Johansen, S. D., Searle, S., Lien, S., Nilsen, F., Jonassen, I., Omholt, S. W., Stenseth, N. C. & Jakobsen, K. S. (2011). The genome sequence of Atlantic cod reveals a unique immune system. *Nature* **477,** 207–210.

Stark, A., Lin, M. F., Kheradpour, P., Pedersen, J. S., Parts, L., Carlson, J. W., Crosby, M. A., Rasmussen, M. D., Roy, S., Deoras, A. N., Ruby, J. G., Brennecke, J., Harvard FlyBase Curators, Berkeley Drosophila Genome Project, Hodges, E., Hinrichs, A. S., Caspi, A., Paten, B., Park, S.-W., Han, M. V., Maeder, M. L., Polansky, B. J., Robson, B. E., Aerts, S., van Helden, J., Hassan, B., Gilbert, D. G., Eastman, D. A., Rice, M., Weir, M., Hahn, M. W., Park, Y., Dewey, C. N., Pachter, L., Kent, W. J., Haussler, D., Lai, E. C., Bartel, D. P., Hannon, G. J., Kaufman, T. C., Eisen, M. B., Clark, A. G., Smith, D., Celniker, S. E., Gelbart, W. & Kellis, M. (2007). Discovery of functional elements in 12 drosophila genomes using evolutionary signatures. *Nature* **450,** 219–232. https://doi.org/10.1038/nature06340

Stuart-Smith, R. D., Bates, A. E., Lefcheck, J. S., Duffy, J. E., Baker, S. C., Thomson, R. J., Stuart-Smith, J. F., Hill, N. A., Kininmonth, S. J., Airoldi, L., Becerro, M. A., Campbell, S. J., Dawson, T. P., Navarrete, S. A., Soler, G. A., Strain, E. M. A., Willis, T. J. & Edgar,

G. J. (2013). Integrating abundance and functional traits reveals new global hotspots of fish diversity. *Nature* **501,** 539–542.
Tarnecki, A. M., Burgos, F. A., Ray, C. L. & Arias, C. R. (2017). Fish intestinal microbiome: diversity and symbiosis unravelled by metagenomics. *Journal of Applied Microbiology* **123,** 2–17. https://doi.org/10.1111/jam.13415
Thomsen, P. F., Kielgast, J., Iversen, L. L., Møller, P. R., Rasmussen, M. & Willerslev, E. (2012). Detection of a diverse marine fish fauna using environmental DNA from seawater samples. *PLoS One* **7,** e41732.
Thurstan, R. H. & Roberts, C. M. (2010). Ecological meltdown in the firth of Clyde, Scotland: two centuries of change in a coastal marine ecosystem. *PLoS One* **5,** e11767.
Thurstan, R. H., Brockington, S. & Roberts, C. M. (2010). The effects of 118 years of industrial fishing on UK bottom trawl fisheries. *Nature Communications* **1,** 15.
Trueman, C. N., MacKenzie, K. M. & Palmer, M. R. (2012). Identifying migrations in marine fishes through stable-isotope analysis. *Journal of Fish Biology* **81,** 826–847.
Turner, C. R., Barnes, M. A., Xu, C. C., Jones, S. E., Jerde, C. L. & Lodge, D. M. (2014). Particle size distribution and optimal capture of aqueous macrobial eDNA. *Methods in Ecology and Evolution* **5,** 676–684.
Uchii, K., Doi, H. & Minamoto, T. (2016). A novel environmental DNA approach to quantify the cryptic invasion of non-native genotypes. *Molecular Ecology Resources* **16,** 415–422.
Ulibarri, R. M., Bonar, S. A., Rees, C., Amberg, J., Ladell, B. & Jackson, C. (2017). Comparing efficiency of American Fisheries Society standard snorkeling techniques to environmental DNA sampling techniques. *North American Journal of Fisheries Management* **37,** 644–651.
Valentini, A., Taberlet, P., Miaud, C., Civade, R., Herder, J., Thomsen, P. F., Bellemain, E., Besnard, A., Coissac, E., Boyer, F. & Gaboriaud, C. (2016). Next-generation monitoring of aquatic biodiversity using environmental DNA metabarcoding. *Molecular Ecology* **25,** 929–942.
Van Neer, W. & Ervynck, A. (2010). Defining "natural" fish communities for fishery management purposes: biological, historical and archaeological approaches. In *Ecologies and Economies in Medieval and Early Modern Europe* (Bruce, S. G., ed), pp. 188–218. Leiden: Brill.
Vellend, M. (2003). Island biogeography of genes and species. *The American Naturalist* **162,** 358–365.
Vellend, M., Lajoie, G., Bourret, A., Múrria, C., Kembel, S. W. & Garant, D. (2014). Drawing ecological inferences from coincident patterns of population and community-level biodiversity. *Molecular Ecology* **23,** 2890–2901. https://doi.org/10.1111/mec.12756
Voegeli, F. A., Smale, M. J., Webber, D. M., Andrade, Y. & O'Dor, R. K. (2001). Ultrasonic telemetry, tracking and automated monitoring technology for sharks. *Environmental Biology of Fishes* **60,** 267–281.
Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature Reviews Genetics*, **10**(1), 57–63.
Weijerman, M., Fulton, E. A., Kaplan, I. C., Gorton, R., Leemans, R., Mooij, W. M. & Brainard, R. E. (2015). An integrated coral reef ecosystem model to support resource management under a changing climate. *PLoS One* **10,** e0144165.
Wheat, R. E., Wang, Y., Byrnes, J. E. & Ranganathan, J. (2013). Raising money for scientific research through crowdfunding. *Trends in Ecology & Evolution* **28,** 71–72. https://doi.org/10.1016/j.tree.2012.11.001
Wheeler, A. & Jones, A. K. G. (1989). *Fishes. Cambridge Manuals in Archaeology*. Cambridge: Cambridge University Press.
Whoriskey, F. G. (2015). The ocean tracking network: a global partnership uses electronic tagging technologies to track the movements of aquatic animals, answer science questions, stimulate new technology development and assist with sustainable development of the ocean. *Development and International Law* **47,** 221–232.
Wilcox, T. M., McKelvey, K. S., Young, M. K., Lowe, W. H. & Schwartz, M. K. (2015). Environmental DNA particle size distribution from brook trout (*Salvelinus fontinalis*). *Conservation Genetics Resources* **7,** 639–641.
Worm, B., Hilborn, R., Baum, J. K., Branch, T. A., Collie, J. S., Costello, C., Fogarty, M. J., Fulton, E. A., Hutchings, J. A., Jennings, S., Jensen, O. P., Lotze, H. K., Mace, P. M.,

McClanahan, T. R., Minto, C., Palumbi, S. R., Parma, A. M., Ricard, D., Rosenberg, A. A., Watson, R. & Zeller, D. (2009). Rebuilding global fisheries. *Science* **325,** 578–585. https://doi.org/10.1126/science.1173146

Xia, J. H., Lin, G., Fu, G. H., Wan, Z. Y., Lee, M., Wang, L., Liu, X. J. & Yue, G. H. (2014). The intestinal microbiome of fish under starvation. *BMC Genomics* **15,** 266. https://doi.org/10.1186/1471-2164-15-266

Yamamoto, S., Minami, K., Fukaya, K., Takahashi, K., Sawada, H., Murakami, H., Tsuji, S., Hashizume, H., Kubonaga, S., Horiuchi, T. & Hongo, M. (2016). Environmental DNA as a 'snapshot' of fish distribution: a case study of Japanese jack mackerel in Maizuru Bay, sea of Japan. *PLoS One* **11,** e0149786.

Zeller, D., Harper, S., Zylich, K. & Pauly, D. (2015). Synthesis of underreported small-scale fisheries catch in Pacific island waters. *Coral Reefs* **34,** 25–39.

Electronic References

Callou, C. (2009). Inventaires archéozoologiques et archéobotaniques de France (Inventaire National Du Patrimoine Naturel). *Muséum National d'Histoire Naturelle* Available at inpn.mnhn.fr/espece/jeudonnees/3471?lg=en

Davis, R., Baumgartner, M., Comeau, A., Cunningham, D., Davies, K., Furlong, A., Johnson, H., L'Orsa, S., Ross, T., Taggart, C. & Whoriskey, F. (2016). Tracking whales on the Scotian Shelf using passive acoustic monitoring on ocean gliders. In Oceans *2016*. Piscataway, NJ: Institute of Electrical and Electronics Engineers Available at www.ieeexplore.ieee.org/document/7761461/authors

Froese, R. & Pauly, D. (Eds) (2017). FishBase. Available at www.fishbase.org.

NASCO (2008) Salmon at Sea – SALSEA – An International Cooperative Research Programme on Salmon at Sea. *NASCO Report*: *SAL 04(5)*. Edinburgh: North Atlantic Salmon Conservation Organization. www.nasco.int/sas/pdf/archive/other_reports/salsea_programme.pdf (accessed 31st October 2017).

OSPAR Convention (2013). *An Assessment of the Ecological Coherence of the OSPAR Network of Marine Protected Areas in 2012*. London: OSPAR Commission Available at www.ospar.org/about/publications?q=An+assessment+of+the+Ecological+coherence+of+the+OSPAR+Network+of+Marine+Protected+Areas+in+2012&a=&y=2013&s=